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(54) **ANTIBODY-MODIFIED CHIMERIC ANTIGEN RECEPTOR MODIFIED T CELL AND USES THEREOF**

(57) The invention relates to a T cell expressing an antibody or comprising the coding sequence of the antibody or an expression vector thereof; the antibody contains an optional signal peptide, an antigen binding sequence and a mutant type Fc segment, wherein the mutant type Fc segment is a Fc segment in which amino acid residues at the 17th site and the 79th site of the IgG4 Fc segment shown by SEQ ID NO: 25 are mutated into E and Q respectively. Preferably, the T cell is a CAR-T cell. The present invention further relates to a treatment application of the T cell in malignant tumors.

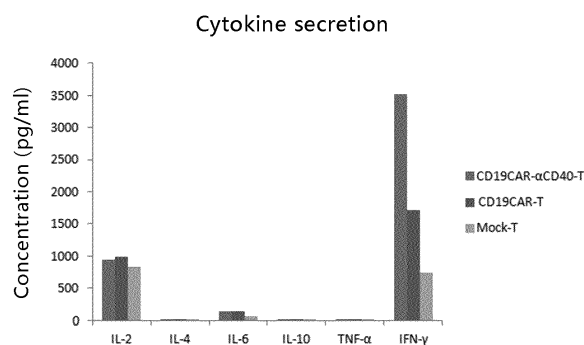


Figure 1

Description

Technical Field

5 **[0001]** The invention relates to antibody-modified chimeric antigen receptor modified T cell and uses thereof.

Background

10 **[0002]** Chimeric antigen receptor T cell (CAR-T) therapy technology is undoubtedly a rising star in the field of tumor immune cell therapy. CAR-T technology includes: splicing the gene sequence of the antibody variable region that recognizes an antigen molecule with the sequence of the intracellular region of a T lymphocyte immune receptor by genetic engineering technology, introducing the linked sequences into lymphocytes by retrovirus or lentiviral vector, transposon or transposase system or directly mRNA transduction, and expressing the fusion proteins on the cell surface. Such T lymphocytes can recognize specific antigens in a non-MHC-restricted manner, which enhances their ability to recognize and kill tumors.

15 **[0003]** The structure of CAR was first proposed by the Eshhar research team of Israeli in 1989. After nearly 30 years of development, T cells modified by the CAR structure have been shown to have good efficacy in tumor immunotherapy. The first-generation CAR receptors contain extracellular fragments that specifically recognize tumor antigens (single-chain variable fragment, scFv), and intracellular activation signals are transmitted by the CD3 ζ signal chain. However, 20 the first-generation CAR receptor lacks the co-stimulatory signal of T cells, which causes T cells to exert only an instant effect, with a short duration in the body and little secretion of cytokines. The second-generation CAR receptor further contains the intracellular domains of a co-stimulatory signaling molecule, including domains of CD28, CD134/OX40, CD137/4-1BB, lymphocyte-specific protein tyrosine kinase (LCK), inducible T-cell co-stimulator (ICOS) and DNAX-activation protein 10 (DAP10) and the like, enhancing the proliferation of T cells and the secretion of cytokines, and 25 increasing IL-2, IFN- γ and GM-CSF. Therefore, the second-generation CAR receptor is not affected by immunosuppression of the tumor microenvironment, and extends AICD (activation induced cell death). The third-generation CAR receptor further fuses a secondary co-stimulatory molecule such as 4-1BB between the co-stimulatory structure CD28 and the ITAM signal chain, thus generating a triple-signal CAR receptor. T cells engineered by the third-generation CAR receptor have better effector function and longer survival time *in vivo*. The commonly used typical CAR-T structure is that of the 30 second-generation CAR receptor, which can be divided into the following four parts: antibody single-chain variable region (scFv) that recognizes a tumor antigen, hinge region, transmembrane region, and intracellular stimulation signal regions. The hinge region of the CAR is responsible for forming the correct conformation and forming dimers. The length and amino acid sequence characteristic of the hinge region determine the spatial conformation of the CAR and its ability to bind to tumor cell surface antigens.

35 **[0004]** At present, CAR-T cells against different targets are undergoing clinical trials for solid tumors treatment, the targets including GD2, FR- α , L1-CAM, HER2, EGFR, EGFRvIII, VEGFR-2, IL-13R α 2, FAP, Mesothelin, c-MET, PSMA, CEA, GPC3, EphA2, MUC1, CAIX (carbonic anhydrase IX), etc. Some clinical trials have relatively good results. For example, in the clinical trial of CAR-T cells against GD2 for the treatment of high-risk neuroblastoma (19 patients), 8 patients had complete tumor regression after reinfusion, 3 patients that did not regress showed a complete response at 40 the 6th week after reinfusion, 1 patients that completely responded still had CAR-T cells after 192 weeks; in the clinical trial of CAR-T cells against HER2 for the treatment of HER2-positive solid tumor (19 cases, of which 16 cases are osteosarcoma), 4 cases maintained a progression-free state for 12 weeks to 14 months, of which 3 cases had tumor regression, 1 case had more than 90% regression. However, compared with hematological tumors, CAR-T treatment for solid tumors generally has poor efficacy, mainly for the following reasons:

45 1. Immunosuppressive microenvironment

[0005] Solid tumor tissue has an immunosuppressive microenvironment, including Treg cells, tumor-associated fibroblasts, bone marrow-derived immature DC cells, M2-type macrophages, and cytokines secreted by them, such as 50 IL-6, IL-10, IDO, VEGF, TGF β , etc. These cells and their secreted cytokines can inhibit the function of T cells directly or indirectly. The immunosuppressive microenvironment of the solid tumor can be affected, therefore improving the survival ability and killing effect of infiltrating CAR-T cells, by methods including destroying tumor microenvironment by radiotherapy and chemotherapy, specifically blocking related signaling pathways by immune checkpoint antibodies (such as PD1/PDL1) or negative immunoregulators (such as small molecule inhibitors of IDO), overall adjusting immune 55 microenvironment by inhibitors of epigenetic modification-related enzymes, over-expressing positive immunoregulatory factors (such as IL-12), and direct targeted removal of tumor stromal cells (such as CAR-T targeting FAP-positive tumor-associated fibroblasts).

2. Lack of suitable CAR-T treatment targets

[0006] Solid tumors are highly heterogeneous, and there are huge differences between solid tumors of different patients, different lesions in the same patient, or different tumor cells in the same lesion. This high degree of heterogeneity has led to the disadvantage of lacking ideal universal and broad-spectrum targets for tumor targeted therapy, which limits the efficacy of CAR-T cells in the treatment of solid tumors. Therefore, in order to make CAR-T cells kill more kinds of tumors, some scholars have proposed the idea of TanCAR by connecting two scFvs that bind to different tumor-associated antigens together to form a new CAR that can simultaneously recognize and bind to two targets, which effectively improves the efficacy of CAR-T cells.

3. It is difficult to reach an effective amount in the body

[0007] T cells need a cluster effect to kill tumor cells, that is, killing one tumor cell requires the cooperation of several T cells. Therefore, only after the effector cells reach a certain number, the tumor cells can be effectively killed. Therefore, T cells can rapidly proliferate upon contacting tumor cells with specific targets, and amplify the killing effect through direct contact and paracrine pathways. CAR-T cells are administered intravenously. In the case of blood tumor, CAR-T cells are very easy to contact tumor cells, leading the number of CAR-T cells rapidly enlarged, or even excessively enlarged to form a cytokine storm; therefore the efficacy is relatively good. However, in the case of solid tumor, CAR-T cells need to reach the tumor site to receive stimulation due to the number of tumor cells in the circulation is limited; therefore it is difficult to achieve an effective amount.

[0008] Therefore, if CAR-T cells, especially those can broadly recognize tumor membrane antigens, express antibodies that directly or indirectly stimulate T cell proliferation and survival, the difficulty in CAR-T treatment for solid tumor can be effectively overcome, and the efficacy can be greatly improved.

SUMMARY

[0009] The present disclosure provides a T cell expressing an antibody or comprising a coding sequence of the antibody or an expression vector thereof, the antibody comprising an optional signal peptide, an antigen binding sequence, and a mutant Fc segment, wherein, the mutant Fc segment is a mutant Fc segment in which the amino acid residues at positions corresponding to positions 17 and 79 of the IgG4 Fc segment shown as SEQ ID NO: 25 are mutated to E and Q, respectively.

[0010] In one or more embodiments, the mutant Fc segment is a mutant IgG4 Fc segment, and its amino acid sequence is preferably as shown in amino acid residues 269-497 of SEQ ID NO: 1, preferably its coding sequence is as shown in nucleotide residues 805-1491 of SEQ ID NO: 2.

[0011] In one or more embodiments, an expression cassette of the antibody is integrated into the T cell genome.

[0012] In one or more embodiments, the signal peptide is a light chain signal peptide, and its amino acid sequence is preferably as shown in amino acid residues 1-20 of SEQ ID NO: 1, preferably its coding sequence is as shown in nucleotide residues 1-60 of SEQ ID NO: 2.

[0013] In one or more embodiments, the antigen binding sequence is derived from an antibody or an antigen-binding fragment thereof that specifically binds to the antigen, such as a single-chain antibody, or from a ligand of a protein that functions in the tumor microenvironment or a fragment thereof that binds to the protein; preferably, the antibody is an agonistic antibody or an inhibitory antibody.

[0014] In one or more embodiments, the agonistic antibody is selected from antibodies directed against one or more of the following antigens: CD28, CD137, CD134, CD40, CD40L, ICOS, HVEM, CD2, CD27, CD30, GITR, LIGHT, DR3, SLAM, CD226, CD80 and CD86.

[0015] In one or more embodiments, the inhibitory antibody is selected from antibodies directed against one or more of the following antigens: PD-1, CTLA4, PDL1, PDL2, PDL3, TIM3, LAG3, CD47, BTLA, TIGIT, CD160, LAIR1, B7-H1, B7-1, VSIR and CD244; preferably, the ligand is a ligand of CD47.

[0016] In one or more embodiments, the agonistic antibody is a CD40 single chain antibody; preferably, the amino acid sequence of the light chain variable region of the CD40 single chain antibody is as shown in amino acid residues 21-146 of SEQ ID NO: 1, and/or the amino acid sequence of the heavy chain variable region of the CD40 single-chain antibody is as shown in amino acid residues 161-268 of SEQ ID NO: 1; preferably, the amino acid sequence of the CD40 single-chain antibody is as shown in amino acid residues 21-268 of SEQ ID NO: 1.

[0017] In one or more embodiments, the inhibitory antibody is a PD-1 single chain antibody; preferably, the amino acid sequence of the light chain variable region of the PD-1 single chain antibody is as shown in amino acid residues 21-131 of SEQ ID NO: 3, and/or the amino acid sequence of the heavy chain variable region of the PD-1 single-chain antibody is as shown in amino acid residues 147-266 of SEQ ID NO: 3; preferably, the amino acid sequence of the PD-1 single-chain antibody is as shown in amino acid residues 21-266 of SEQ ID NO: 3.

[0018] In one or more embodiments, the amino acid sequence of the CD47 ligand is shown as amino acid residues 21-138 of SEQ ID NO: 5.

[0019] In one or more embodiments, the coding sequence of the light chain variable region of the CD40 single-chain antibody is as shown in nucleotide residues 60-438 of SEQ ID NO: 2, and/or the coding sequence of the heavy chain variable region thereof may be as shown in nucleotide residues 481-804 of SEQ ID NO: 2; preferably, the coding sequence of the CD40 single chain antibody is shown as nucleotide residues 60-804 of SEQ ID NO: 2.

[0020] In one or more embodiments, the coding sequence of the light chain variable region of the PD-1 single-chain antibody is as shown in nucleotide residues 60-393 of SEQ ID NO: 4, and/or the coding sequence of the heavy chain variable region thereof is as shown in nucleotide residues 439-798 of SEQ ID NO: 4; preferably, the coding sequence of the PD-1 single chain antibody is shown as nucleotide residues 60-798 of SEQ ID NO: 4.

[0021] In one or more embodiments, the coding sequence of the CD47 ligand is shown as nucleotide residues 60-414 of SEQ ID NO: 6.

[0022] In one or more embodiments, the antibody is a CD40 antibody, PD-1 antibody, or CD47 antibody.

[0023] In one or more embodiments, the amino acid sequence of the CD40 antibody is as shown in amino acid residues 21-497 of SEQ ID NO: 1, or as shown in SEQ ID NO: 1, the amino acid sequence of the PD-1 antibody is as shown in amino acid residues 21-495 of SEQ ID NO: 3, or as shown in SEQ ID NO: 3, the amino acid sequence of the CD47 antibody is as shown in amino acid residues 21-367 of SEQ ID NO: 5, or as shown in SEQ ID NO: 5.

[0024] In one or more embodiments, the coding sequence of the antibody is as shown in nucleotide residues 60-1491 of SEQ ID NO: 2, or as shown in SEQ ID NO: 2; or is as shown in nucleotide residues 60-1485 of SEQ ID NO: 4, or as shown in SEQ ID NO: 4; or is as shown in nucleotide residues 60-1104 of SEQ ID NO: 6, or as shown in SEQ ID NO: 6.

[0025] In one or more embodiments, the T cell is a CAR-T cell expressing a chimeric antigen receptor, wherein the expression cassette of the antibody and the expression cassette of the chimeric antigen receptor are integrated into the genome of the T cell.

[0026] In one or more embodiments, the chimeric antigen receptor recognizes, targets, or specifically binds to one or more of the following antigens: CD19, CD20, CEA, GD2, FR, PSMA, PMEL, CA9, CD171/L1-CAM, IL-13R α 2, MART-1, ERBB2, NY-ESO-1, MAGE family proteins, BAGE family proteins, GAGE family proteins, AFP, MUC1, CD22, CD23, CD30, CD33, CD44v7/8, CD70, VEGFR1, VEGFR2, IL-11R α , EGP-2, EGP-40, FBP, GD3, PSCA, FSA, PSA, HMGA2, fetal acetylcholine receptor, LeY, EpCAM, MSLN, IGFR1, EGFR, EGFRvIII, ERBB3, ERBB4, CA125, CA15-3, CA19-9, CA72-4, CA242, CA50, CYFRA21-1, SCC, AFU, EBV-VCA, POA, β 2-MG and PROGRP; preferably the chimeric antigen receptor is those recognizes, targets, or specifically binds to CD19, mesothelin, EGFR, mucin or ErbB receptor family.

[0027] In one or more embodiments, the chimeric antigen receptor contains an optional signal peptide sequence, antigen recognition region, hinge region, transmembrane region, intracellular co-stimulatory signal domain and intracellular signal domain; wherein, the signal peptide is selected from the group consisting of a CD8 signal peptide, a CD28 signal peptide, a CD4 signal peptide and a light chain signal peptide; the antigen recognition region is an amino acid sequence that recognizes, targets or specifically binds to the target antigen; and the hinge region is selected from the group consisting of a extracellular hinge region of CD8, a hinge region of IgG1 Fc CH2CH3, a IgD hinge region, a extracellular hinge region of CD28, a hinge region of IgG4 Fc CH2CH3 and a extracellular hinge region of CD4, preferably the hinge region is 50 amino acid residues or more in length, more preferably 80 amino acid residues or more in length; preferably, the hinge region is a CD8 α hinge region or a hinge region of IgG4 Fc CH2CH3; the transmembrane region is selected from the group consisting of a transmembrane region of CD28, a transmembrane region of CD8, a transmembrane region of CD3 ζ , a transmembrane region of CD134, a transmembrane region of CD137, a transmembrane region of ICOS and a transmembrane region of DAP10; preferably a transmembrane region of CD8 or a transmembrane region of CD28; the intracellular co-stimulatory signal domain is the intracellular domain of a co-stimulatory signal molecule, which is selected from the group consisting of a intracellular domains of CD28, CD134/OX40, CD137/4-1BB, lymphocyte-specific protein tyrosine kinase, a inducible T cell co-stimulatory factor and a DNAX activating protein 10, preferably a intracellular domain of CD137/4-1BB or a intracellular domain of CD28; and/or the intracellular signal domain is a intracellular signal domain of CD3 ζ or a intracellular signal domain of Fc γ R1, preferably a intracellular signal domain of CD3 ζ .

[0028] In one or more embodiments, the amino acid sequence of the signal peptide is as shown in amino acid residues 1-21 of SEQ ID NO: 7, or amino acid residues 1-22 of SEQ ID NO: 9, or amino acid residues 1-20 of SEQ ID NO: 11; the antigen recognition region is a single-chain antibody that recognizes, targets, or specifically binds to CD19, mesothelin, EGFR, or mucin, or consists of the amino acid sequence that recognizes, targets, or specifically binds to the ErbB receptor family; the amino acid sequence of the hinge region is as shown in amino acid residues 264-308 of SEQ ID NO: 7, or amino acid residues 273-500 of SEQ ID NO: 9, or amino acid residues 264-318 of SEQ ID NO: 17; the amino acid sequence of the transmembrane region is as shown in amino acid residues 309-332 of SEQ ID NO: 7, or amino acid residues 501-528 of SEQ ID NO: 9, or amino acid residues 319-344 of SEQ ID NO: 17; the amino acid sequence of the intracellular co-stimulatory signal domain is as shown in amino acid residues 333-374 of SEQ ID NO: 7, or amino acid residues 529-569 of SEQ ID NO: 9; and/or the amino acid sequence of the intracellular signal domain is as shown

in amino acid residues 375-486 of SEQ ID NO: 7.

[0029] In one or more embodiments, the coding sequence of the signal peptide is as shown in the nucleotide residues 1-63 of SEQ ID NO: 8, or the nucleotide residues 1-66 of SEQ ID NO: 10, or the nucleotide residues 1-60 of SEQ ID NO: 12; the coding sequence of the hinge region is as shown in nucleotide residues 790-924 of SEQ ID NO: 8, or nucleotide residues 817-1500 of SEQ ID NO: 10, or nucleotide residues 790-954 of SEQ ID NO: 18; the coding sequence of the transmembrane region is as shown in the nucleotide residues 925-996 of SEQ ID NO: 8, or the nucleotide residues 1501-1584 of SEQ ID NO: 10, or the nucleotide residues 955-1032 of SEQ ID NO: 18; the coding sequence of the intracellular co-stimulatory signal domain is as shown in the nucleotide residues 997-1122 of SEQ ID NO: 8, or the nucleotide residues 1585-1707 of SEQ ID NO: 10; and/or the coding sequence of the intracellular signal domain is as shown in the nucleotide residues 1123-1458 of SEQ ID NO: 8.

[0030] In one or more embodiments, the amino acid sequence of the light chain variable region of the single-chain antibody that recognizes, targets or specifically binds to CD19 may be as shown in amino acid residues 22-128 of SEQ ID NO: 7, and/or the amino acid sequence of the heavy chain variable region thereof may be as shown in amino acid residues 144-263 of SEQ ID NO: 7; preferably, the amino acid sequence of the single-chain antibody is as shown in amino acid residues 22-263 of SEQ ID NO: 7.

[0031] In one or more embodiments, the single-chain antibody that recognizes, targets or specifically binds to mesothelin antigen is a single-chain antibody directed against Region I or III of mesothelin, preferably the single-chain antibody directed against Region III of mesothelin; preferably, the amino acid sequence of the light chain variable region of the anti-mesothelin Region III single-chain antibody is as shown in amino acid residues 23-146 of SEQ ID NO: 9, and/or the amino acid sequence of the heavy chain variable region of the anti-mesothelin Region III single-chain antibody is as shown in amino acid residues 162-272 of SEQ ID NO: 9; preferably, the amino acid sequence of the single chain antibody that recognizes, targets or specifically binds to mesothelin antigen is as shown in amino acid residues 23-272 of SEQ ID NO: 9.

[0032] In one or more embodiments, the antigen recognition region that recognizes, targets or specifically binds to the ErbB receptor family contains a fusion protein of natural TIE and Herin; wherein, the TIE consists of 7 amino acids at the N-terminus of human transcription growth factor α (TGF α) and 48 amino acids at the C-terminus of epidermal growth factor (EGF), preferably, the amino acid sequence of TIE is as shown in amino acid residues 23-77 of SEQ ID NO: 13; Herin is the 79 amino acids encoded by intron 8 in Herstatin, preferably, the amino acid sequence of Herin is as shown in amino acid residues 93-171 of SEQ ID NO: 13; preferably, the antigen recognition region is as shown in amino acid residues 23-171 of SEQ ID NO: 13.

[0033] In one or more embodiments, the amino acid sequence of the light chain variable region and the amino acid sequence of the heavy chain variable region of the single-chain antibody that recognizes, targets or specifically binds to mucin antigen are derived from an antibody against the amino acid sequence of the membrane-proximal end of Mucl, preferably, the amino acid sequence of the membrane-proximal end of Mucl is as shown in SEQ ID NO: 24; preferably, the amino acid sequence of the light chain variable region of the single chain antibody is as shown in amino acid residues 23-133 of SEQ ID NO: 15, and/or the amino acid sequence of the heavy chain variable region is as shown in amino acid residues 149-269 of SEQ ID NO: 15; preferably, the amino acid sequence of the single chain antibody is as shown in amino acid residues 23-269 of SEQ ID NO: 15.

[0034] In one or more embodiments, the antigen recognition region that recognizes, targets, or specifically binds to EGFR is a single chain antibody formed by the light chain variable region and the heavy chain variable region of an antibody specific for EGFR; preferably, the amino acid sequence of the light chain variable region of the single-chain antibody is as shown in amino acid residues 23-129 of SEQ ID NO: 17, and/or the amino acid sequence of the heavy chain variable region is as shown in amino acid residues 145-263 of SEQ ID NO: 17; preferably, the amino acid sequence of the single chain antibody is as shown in amino acid residues 23-263 of SEQ ID NO: 17.

[0035] In one or more embodiments, the chimeric antigen receptor contains, in the order from the N-terminus to the C-terminus, an optional signal peptide sequence, antigen recognition region, CD8 α hinge region or IgG4 CH2CH3 hinge region, CD8 transmembrane region or CD28 transmembrane region, 4-1BB or CD28 intracellular domain and CD3 ζ intracellular signal domain.

[0036] In one or more embodiments, the chimeric antigen receptor is selected from the group consisting of:

(1) a chimeric antigen receptor targeting CD19, with the amino acid sequence thereof is as shown in amino acid residues 22-486 of SEQ ID NO: 7, or as shown in SEQ ID NO: 7, and the coding sequence thereof is preferably as shown in nucleotide residues 64-1458 of SEQ ID NO: 8, or as shown in SEQ ID NO: 8;

(2) a chimeric antigen receptor targeting mesothelin, with the amino acid sequence thereof is as shown in amino acid residues 23-681 of SEQ ID NO: 9, or as shown in SEQ ID NO: 9, the coding sequence thereof is preferably as shown in nucleotide residues 67-2043 of SEQ ID NO: 10, or as shown in SEQ ID NO: 10, or the amino acid sequence thereof is as shown in amino acid residues 21-679 of SEQ ID NO: 11, or as shown in SEQ ID NO: 11, the coding sequence thereof is preferably as shown in nucleotide residues 61-2037 of SEQ ID NO: 12, or as shown in SEQ ID NO: 12.

NO: 12;

(3) an antigen recognition region targeting ErbB family, with the amino acid sequence thereof is as shown in amino acid residues 23-580 of SEQ ID NO: 13, or as shown in SEQ ID NO: 13, and the coding sequence thereof is preferably as shown in nucleotide residues 67-1740 of SEQ ID NO: 14, or as shown in SEQ ID NO: 14;

(4) a chimeric antigen receptor targeting mucin, with the amino acid sequence thereof is as shown in amino acid residues 23-678 of SEQ ID NO: 15, or as shown in SEQ ID NO: 15, and the coding sequence thereof is preferably as shown in nucleotide residues 67-2034 of SEQ ID NO: 16, or as shown in SEQ ID NO: 16; and

(5) a chimeric antigen receptor targeting EGFR, with the amino acid sequence thereof is as shown in amino acid residues 23-497 of SEQ ID NO: 17, or as shown in SEQ ID NO: 17, and the coding sequence thereof is preferably as shown in nucleotide residues 67-1491 of SEQ ID NO: 18, or as shown in SEQ ID NO: 18.

[0037] The present invention also provides an antibody, comprising an optional signal peptide, an antigen binding sequence, and a mutant Fc segment, wherein, the mutant Fc segment is a mutant Fc segment in which the amino acid residues at positions corresponding to positions 17 and 79 of the IgG4 Fc segment shown as SEQ ID NO: 25 are mutated to E and Q, respectively.

[0038] In one or more embodiments, the mutant Fc segment is a mutant IgG4 Fc segment, and its amino acid sequence is preferably as shown in amino acid residues 269-497 of SEQ ID NO: 1, preferably its coding sequence is as shown in nucleotide residues 805-1491 of SEQ ID NO: 2.

[0039] In one or more embodiments, the signal peptide is a light chain signal peptide, and its amino acid sequence is preferably as shown in amino acid residues 1-20 of SEQ ID NO: 1, preferably its coding sequence is as shown in nucleotide residues 1-60 of SEQ ID NO: 2.

[0040] In one or more embodiments, the antigen binding sequence is derived from an antibody or an antigen-binding fragment thereof that specifically binds to the antigen, such as a single-chain antibody, or from a ligand of a protein that functions in the tumor microenvironment or a fragment thereof that binds to the protein; preferably, the antibody is an agonistic antibody or an inhibitory antibody; preferably, the agonistic antibody is selected from antibodies directed against one or more of the following antigens: CD28, CD137, CD134, CD40, CD40L, ICOS, HVEM, CD2, CD27, CD30, GITR, LIGHT, DR3, SLAM, CD226, CD80 and CD86; preferably, the inhibitory antibody is selected from antibodies directed against one or more of the following antigens: PD-1, CTLA4, PDL1, PDL2, PDL3, TIM3, LAG3, CD47, BTLA, TIGIT, CD160, LAIR1, B7-H1, B7-1, VSIR and CD244; preferably, the ligand is a ligand of CD47.

[0041] In one or more embodiments, the agonistic antibody is a CD40 single chain antibody; preferably, the amino acid sequence of the light chain variable region of the CD40 single chain antibody is as shown in amino acid residues 21-146 of SEQ ID NO: 1, and/or the amino acid sequence of the heavy chain variable region of the CD40 single-chain antibody is as shown in amino acid residues 161-268 of SEQ ID NO: 1; preferably, the amino acid sequence of the CD40 single-chain antibody is as shown in amino acid residues 21-268 of SEQ ID NO: 1.

[0042] In one or more embodiments, the inhibitory antibody is a PD-1 single chain antibody; preferably, the amino acid sequence of the light chain variable region of the PD-1 single chain antibody is as shown in amino acid residues 21-131 of SEQ ID NO: 3, and/or the amino acid sequence of the heavy chain variable region of the PD-1 single-chain antibody is as shown in amino acid residues 147-266 of SEQ ID NO: 3; preferably, the amino acid sequence of the PD-1 single-chain antibody is as shown in amino acid residues 21-266 of SEQ ID NO: 3.

[0043] In one or more embodiments, the amino acid sequence of the CD47 ligand is shown as amino acid residues 21-138 of SEQ ID NO: 5.

[0044] In one or more embodiments, the coding sequence of the light chain variable region of the CD40 single-chain antibody is as shown in nucleotide residues 60-438 of SEQ ID NO: 2, and/or the coding sequence of the heavy chain variable region thereof may be as shown in nucleotide residues 481-804 of SEQ ID NO: 2; preferably, the coding sequence of the CD40 single chain antibody is shown as nucleotide residues 60-804 of SEQ ID NO: 2.

[0045] In one or more embodiments, the coding sequence of the light chain variable region of the PD-1 single-chain antibody is as shown in nucleotide residues 60-393 of SEQ ID NO: 4, and/or the coding sequence of the heavy chain variable region thereof is as shown in nucleotide residues 439-798 of SEQ ID NO: 4; preferably, the coding sequence of the PD-1 single chain antibody is shown as nucleotide residues 60-798 of SEQ ID NO: 4.

[0046] In one or more embodiments, the coding sequence of the CD47 ligand is shown as nucleotide residues 60-414 of SEQ ID NO: 6.

[0047] In one or more embodiments, the antibody is a CD40 antibody, PD-1 antibody, or CD47 antibody.

[0048] In one or more embodiments, the amino acid sequence of the CD40 antibody is as shown in amino acid residues 21-497 of SEQ ID NO: 1, or as shown in SEQ ID NO: 1, the amino acid sequence of the PD-1 antibody is as shown in amino acid residues 21-495 of SEQ ID NO: 3, or as shown in SEQ ID NO: 3, the amino acid sequence of the CD47 antibody is as shown in amino acid residues 21-367 of SEQ ID NO: 5, or as shown in SEQ ID NO: 5.

[0049] In one or more embodiments, the coding sequence of the antibody is as shown in nucleotide residues 60-1491 of SEQ ID NO: 2, or as shown in SEQ ID NO: 2; or is as shown in nucleotide residues 60-1485 of SEQ ID NO: 4, or as

shown in SEQ ID NO: 4; or is as shown in nucleotide residues 60-1104 of SEQ ID NO: 6, or as shown in SEQ ID NO: 6.

[0050] Also provided is a nucleic acid sequence, selected from the group consisting of the coding sequences of the antibodies described herein or complementary sequences thereof.

[0051] Also provided is a nucleic acid construct containing the nucleic acid sequence described herein; preferably, the nucleic acid construct is an expression cassette or vector.

[0052] In one or more embodiments, the nucleic acid construct is an expression vector or an integration vector for incorporating the expression cassette into the genome of a host cell.

[0053] In one or more embodiments, the integration vector is an integration vector comprising a promoter, the coding sequence of the antibody described herein, and a polyA tailing signal sequence, in operable linkage between 5'LTR and 3'LTR, and not comprising a transposase coding sequence.

[0054] Also provided is a composition comprising the vector described herein and an optional transfection reagent; preferably, the composition comprises the integration vector described herein and an integration vector for incorporating an expression cassette of a chimeric antigen receptor into the genome of a host cell; preferably, the chimeric antigen receptor is as defined in any of the embodiments herein.

[0055] In one or more embodiments, in the composition, the mass ratio of the integration vector for incorporating the expression cassette of the chimeric antigen receptor into the genome of the host cell and the integration vector for incorporating the expression cassette of the antibody described herein into the genome of the host cell is 1-7:1-7, such as 1-5:1-5, preferably 1-3:1-3, more preferably 1-2:1-2, and even more preferably 1-2:1.

[0056] Also provided is a kit comprising the vector described herein and an optional transfection reagent; preferably, the kit comprises the integration vector for incorporating the expression cassette of the antibody described herein into the genome of the host cell and an integration vector for incorporating an expression cassette of a chimeric antigen receptor into the genome of a host cell; preferably, the chimeric antigen receptor is as defined in any of the embodiments herein;

[0057] In one or more embodiments, the kit contains the composition described herein.

[0058] Also provided is a pharmaceutical composition containing the T cells described herein or the T cells and the antibodies described herein expressed by the T cells.

[0059] Also provided is a host cell containing the nucleic acid sequence or nucleic acid construct described herein.

[0060] Also provided is the use of the T cells, antibodies, nucleic acid sequences, nucleic acid constructs, and host cells described herein in the preparation of a medicament for treating or preventing malignant tumors.

[0061] In one or more embodiments, the malignant tumor is selected from the group consisting of: acute B-lymphocytic leukemia, chronic B-lymphocytic leukemia, mantle cell lymphoma, non-Hodgkin's lymphoma, and multiple myeloma; or is a malignant tumor in which a cancer cell abnormally expresses mesothelin, at least one EGFR family member protein, a Mucl antigen, EGFR and/or CD47 on cell surface; or is a malignant tumor mediated by CD40 or PD1.

[0062] Also provided is a method for preparing T cells, including the step of transfecting the T cells with the following vectors:

(1) the vector that is for transferring the expression cassette of the chimeric antigen receptor into the genome of the T cell and contains a transposase coding sequence, and

(2) the vector that is for transferring the expression cassette of the antibody into the genome of the T cell and contains no transposase coding sequence;

preferably, the mass ratio of the vectors of (1) and (2) is 1-7:1-7, such as 1-5:1-5, preferably 1-3:1-3, more preferably 1-2:1-2, more preferably 1-2:1.

BRIEF DESCRIPTION OF DRAWINGS

[0063]

Figure 1: Comparison of CD19CAR-secretion, changes of secretions of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ upon the stimulation of CD19 antigen.

Figure 2: Proliferation detection of CD19CAR T cells and CD19CAR- α CD40 T cells.

Figure 3: The therapeutic effects of CD19CAR T cells, CD19CAR- α CD40-wt T, and CD19CAR- α CD40 T cells on the Raji-luc mouse xenograft model.

Figure 4: The killing of mesoCAR- α CD40 T cells on cervical cancer cells Hela, ovarian cancer cells SK-OV-3 and gastric cancer cells HGC-27.

Figure 5: Changes of secretions of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ of mesoCAR- α CD40 upon the stimulation of CD19 antigen.

Figure 6: Proliferation detection of mesoCAR T cells and mesoCAR- α CD40 T cells.

Figure 7: The therapeutic effects of mesoCAR T cells and mesoCAR- α CD40-T cells on the SK-OV-3 ovarian cancer mouse xenograft model.

Figure 8: Comparison of the proliferation rate of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells.

Figure 9A-9D: Phenotype analysis of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells; 9A represents the aging phenotype CD40, 9B and 9C represent the activated phenotype CD69 and CD107 α respectively, 9D represents the memory phenotype.

Figure 10: Comparison of the killing by EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells, including human liver cancer cells HCCLM3, human lung degenerative cancer cells Calu-6 and human non-small cell lung cancer H23.

Figure 11: Changes of secretions of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells upon the stimulation of EGFR antigen.

Figure 12: Changes in fluorescence values of the tumor cells at different days after treating mice with EHCAR-EK-28TIZ T cells, EHCAR-EK-28TIZ- α CD40-wt T cells, EHCAR-EK-28TIZ- α CD40 T cells, Mock-T cells and PBS blank control.

Figure 13: Comparison of the proliferation rate of Muc1CAR T cells and Muc1CAR- α CD40 T cells.

Figures 14A-14B: Phenotype analysis of Muc1CAR T cells and Muc1CAR- α CD40 T cells, Figure 14A shows the aging phenotype PD1, LAG3 and activated phenotype CD25, Figure 14B shows the memory phenotype.

Figure 15: Comparison of the killing by Muc1CAR T cells and Muc1CAR- α CD40 T cells, including human liver cancer cells HCCLM3 and human non-small cell lung cancer H23.

Figure 16: Changes of secretions of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ of Muc1CAR T cells and Muc1CAR- α CD40 T cells upon the stimulation of Muc1 antigen.

Figure 17: Changes in fluorescence values of the tumor cells at different days after treating mice with Muc1CAR T cells, Muc1CAR- α CD40-wt T cells, Muc1CAR- α CD40 T cells, Mock-T cells and PBS blank control.

Figure 18: Detection of the killing effect of CD19CAR-anti PD1 pluripotent T cells.

Figure 19: CD19CAR-anti PD1 pluripotent T cells can enhance the killing activity of T cells *in vitro*.

Figure 20: Detection of the killing effect of CD19CAR-anti PD1 T cell *in vivo*.

Figure 21: The killing of mesoCAR-antiPD1 T cells on cervical cancer cells Hela, ovarian cancer cells SK-OV-3 and gastric cancer cells HGC-27.

Figure 22: Changes of secretions of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ of mesoCAR-antiPD1 upon the stimulation of mesothelin antigen.

Figure 23: The therapeutic effects of meso3CAR T cells and mesoCAR-antiPD1 T cells on the SK-OV-3 ovarian cancer xenograft mouse model.

Figure 24: Comparison of the proliferation rate of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells.

Figure 25A-25D: Phenotype analysis of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells; 25A represents the aging phenotype PD1, 25B and 25C represent the activated phenotype CD69 and CD107 α respectively, 25D represents the memory phenotype.

Figure 26: Comparison of the killing by EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells, including human liver cancer cells HCCLM3, human liver cancer cells Hep3B and human non-small cell lung cancer H23.

Figure 27: Changes of secretions of cytokines IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells upon the stimulation of EGFR antigen.

Figure 28: Changes in fluorescence values of the tumor cells at different days after treating mice with EHCAR-EK-28TIZ T cells, EHCAR-EK-28TIZ-antiPD1-wt T cells, EHCAR-EK-28TIZ-antiPD1 T cells, Mock-T cells and PBS blank control.

Figure 29A-29D: Muc1CAR-anti PD1 pluripotent T cells can enhance the killing activity of T cells *in vitro*. 29A: Flow cytometry detection of marker CD107 α that indirectly reflects the T cell killing activity, activation marker CD25 and depletion marker LAG3, 29B: flow cytometry detection of marker proteins CD45RO, CD62L and CCR7 reflecting the T cell memory phenotype. 29C: Flow cytometry detection of the ratio of CD3/CD4/CD8 of T cells. 29D: Detection, through multi-factor detection kit, of the changes in IL-2, IL-4, IL-6, IL-10, TNF- α and IFN- γ cytokines of Muc1CAR-anti PD1 multipotent T cells, Muc1CAR-T cells and Mock T cells upon stimulation by Muc1 antigen.

Figure 30: Detection of the killing effect of Muc1CAR-anti PD1 pluripotent T cells.

Figure 31: *In vivo* function study of Muc1CAR T cells that expresses PD-1 antibody.

Figure 32: Flow cytometry detection of CD47 expression in Mock T cells, EGFR-CAR T cells, and α CD47-EGFR-CAR T cells.

Figure 33: Killing of different tumor cells by α CD47-EGFR-CAR T cells.

Figure 34: α CD47-EGFR-CAR T cell supernatant was co-cultured with different tumor cells to block CD47 on the surface of the tumor cells.

Figure 35: Blocking CD47 on the surface of the tumor cells can enhance the phagocytosis of macrophages to the tumor cells.

Figure 36: Anti-tumor effect of α CD47-EGFR-CAR T cell in mice *in vivo*.

Figure 37: Flow cytometry detection of CD47 expression in Mock, Meso3CAR and α CD47-Meso3CAR T cells.

Figure 38: Killing of tumor cell line by α CD47-Meso3CAR T cells.

Figure 39: α CD47-Meso3CAR T cell supernatant was co-cultured with tumor cells to block CD47 on the surface of the tumor cells.

Figure 40: Blocking CD47 on the surface of the tumor cells can enhance the phagocytosis of macrophages to the tumor cells.

Figure 41: Anti-tumor effect of α CD47-Meso3CAR T cell *in vivo*.

[0064] In addition, the ordinates "count" in the Figures are "number of cells".

DETAILED DESCRIPTION

[0065] It should be understood that, within the scope of the present disclosure, the above technical features of the present disclosure and the technical features specifically described in the following (e.g., Examples) can be combined with each other, thereby forming preferred technical solution(s).

[0066] Some terms involved in the present disclosure will be explained below.

[0067] In the present disclosure, the term "expression cassette" refers to the complete elements required to express a gene, including an operably linked promoter and gene coding sequence.

[0068] The term "coding sequence" is defined herein as a part of a nucleic acid sequence that directly determines the amino acid sequence of its protein product (e.g., CAR, single chain antibody, hinge region, and transmembrane region). The boundaries of a coding sequence are usually determined by a ribosome binding site closely adjacent to the upstream of open reading frame at mRNA 5' end (for prokaryotic cells) and a transcriptional termination sequence closely adjacent to the downstream of the open reading frame at mRNA 3' end. The coding sequence can include, but is not limited to, DNA, cDNA and recombinant nucleic acid sequences.

[0069] The term "Fc" refers to the crystallizable fragment of an antibody, which means a peptide fragment located at the terminal of the "Y" handle structure of the antibody, comprising peptide segments of antibody heavy chain constant regions CH2 and CH3, which is the part of an antibody that interacts with effector molecule or cell.

[0070] The term "co-stimulating molecule" refers to a molecule that exists on the surface of an antigen-presenting cell, which is capable of binding to co-stimulating molecular receptors on Th cells to generate synergistic stimulation signals. The proliferation of lymphocyte not only requires binding of the antigen, but also the receiving of the co-stimulating molecule signal. The transmission of co-stimulation signal to T cell is mainly by the binding of co-stimulating molecules CD80 and CD86 expressed on the surface of an antigen-presenting cell to CD28 molecule on the surface of T cells. The receiving of co-stimulation signal by B cells can be mediated by common pathogen components such as LPS, or by complement component, or by CD40L on activated antigen-specific Th cell surface.

[0071] The term "linker" or hinge is a polypeptide fragment that links different proteins or polypeptides, and the purpose of which is to keep the independent spatial conformation of the linked protein or polypeptide to maintain the function or activity of the protein or polypeptide. Exemplary linkers include those containing G and/or S, and, for example, Furin 2A peptide.

[0072] The term "specific binding" refers to the reaction between an antibody or antigen binding fragment and an antigen which it recognizes. In certain embodiments, an antibody specifically binding to a certain antigen (or an antibody that is specific to a certain antigen) means that the antibody binds to the antigen with an affinity (K_d) of less than about 10^{-5} M, such as less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M or less. "Specific recognition" or "targeting" has a similar meaning.

[0073] The term "a pharmaceutically acceptable excipient" refers to a carrier and/or an excipient that are pharmacologically and/or physiologically compatible with a subject and active ingredient(s), which is well known in the art (see, for example, Remington's Pharmaceutical Sciences, Gennaro AR Ed., 19th edition, Pennsylvania: Mack Publishing Company, 1995), including but not limited to, pH adjusting agent, surfactant, adjuvant, ion strength enhancer. For example, the pH adjusting agent includes, but is not limited to, phosphate buffer; the surfactant includes, but is not limited to, cationic, anionic or non-ionic surfactant, such as Tween-80; the ion strength enhancer includes, but is not limited to, sodium chloride.

[0074] The term "an effective amount" refers to a dosage amount that can treat, prevent, reduce and/or alleviate the disease or condition of the present invention in a subject.

[0075] The term "disease and/or condition" refers to a physical state of the subject, wherein the physical state is related to the diseases and/or conditions of the disclosure.

[0076] The term "subject" or "patient" may refer to a patient or other animals, particularly a mammal, such as a human, a dog, a monkey, a cow, a horse and the like, that receives the pharmaceutical composition of the invention for treating, preventing, reducing and/or alleviating the diseases or conditions of the present invention.

[0077] "Chimeric antigen receptor" (CAR) is an artificially modified receptor which can anchor the specific molecules

(such as antibodies) recognizing tumor cell surface antigens to immune cells (such as T cells), so that the immune cells can recognize tumor antigens or virus antigens and kill tumor cells or virus-infected cells. CARs generally in turn include an optional signal peptide, a polypeptide (such as a single chain antibody) that binds to a tumor cell membrane antigen, a hinge region, a transmembrane region, and an intracellular signal region. In general, the polypeptide that binds to a tumor cell membrane antigen can bind membrane antigens widely expressed by tumor cells with moderate affinity. The polypeptide that binds to a tumor cell membrane antigen may be a natural polypeptide or a synthetic polypeptide; preferably, the synthetic polypeptide is a single chain antibody or a Fab fragment.

[0078] The term "single chain antibody" (scFv) refers to an antibody fragment formed by linking the amino acid sequences of antibody light chain variable region (V_L region) and heavy chain variable region (V_H region) with hinge, which has antigen-binding ability. In certain embodiments, a single chain antibody of interest (scFv) is derived from the antibodies of interest. Antibodies of interest may be human antibodies, including human mouse chimeric antibodies and humanized antibodies. Antibodies may be secretory antibodies or membrane-anchored antibodies; preferably membrane-anchored antibodies.

[0079] The term "operably linked" or "in operably linkage" refers to DNA regulatory sequences (e.g., enhancers, promoters, etc.) linked to the coding sequence of the protein of interest in a manner that allows the coding sequence to be expressed.

1. Mutant Fc segment

[0080] Studies have shown that the IgG4 Fc segments of PD-1 inhibitory antibodies and CD40 agonistic antibodies are easily recognized and phagocytized by monocytes/macrophages. In the present disclosure, the IgG4 Fc segments are modified by base mutation to make the antibodies expressed by T cells themselves can function well without causing ADCC effect.

[0081] In particular, the exemplary IgG4 Fc segment herein has the sequence shown in amino acid residues 269-497 of SEQ ID NO: 1, wherein, compared with the wild-type IgG4 Fc segment (SEQ ID NO: 25), the IgG4 Fc segment herein has a mutation from L to E at position 17, and a mutation from N to Q at position 79.

[0082] The present disclosure also includes Fc segments of other types of antibodies or immunoglobulins, with the mutation of the amino acid residue at position corresponding to position 17 of IgG4 Fc (SEQ ID NO: 25) to E and the mutation of the amino acid residue at position corresponding to position 79 to Q. The types of antibodies or immunoglobulins (Ig) include, but are not limited to, IgM, IgD, IgG, IgA, and IgE, which are well known in the art, wherein the IgG includes IgG1, IgG2, IgG3, and IgG4; IgA includes IgA1 and IgA2. Therefore, in certain embodiments, the present disclosure uses a mutant antibody Fc segment, wherein, the amino acid residue on the Fc segment at the position corresponding to the position 17 of IgG4 Fc segment shown in SEQ ID NO: 25 is E, and the amino acid residue at the position corresponding to position 79 is Q. In certain embodiments, the present disclosure uses mutant Fc segments of membrane-anchored Ig.

[0083] In certain embodiments, the present disclosure uses the Fc segment as shown in amino acid residues 269-497 of SEQ ID NO: 1, and its exemplary coding sequence may be as shown in nucleotide residues 805-1491 of SEQ ID NO: 2.

2. Antigen binding sequence

[0084] As used herein, the term "antigen-binding sequence" includes antibodies or antigen-binding fragments that specifically bind to antigens, such as single-chain antibodies, and also includes ligands of proteins that function in the tumor microenvironment or fragments thereof that bind to the proteins.

[0085] The antibodies expressed by CAR-T cells suitable for the present disclosure may be various antibodies used in tumor therapy, including agonistic antibodies and inhibitory antibodies.

[0086] The agonistic antibodies that can be used in the present disclosure may be various agonistic antibodies well known in the art, including but not limited to antibodies against one or more of the following antigens: CD28, CD137, CD134, CD40, CD40L, ICOS, HVEM, CD2, CD27, CD30, GITR, LIGHT, DR3, SLAM, CD226, CD80 and CD86. In certain embodiments, the agonistic antibody used in the present disclosure is an antibody against CD40. Preferably, the CD40 antibody is a single chain antibody. The amino acid sequence of the light chain variable region (V_L region) of an exemplary CD40 single chain antibody is as shown in amino acid residues 21-146 of SEQ ID NO: 1, an exemplary coding sequence thereof is as shown in nucleotide residues 60-438 of SEQ ID NO: 2; the amino acid sequence of the heavy chain variable region of an exemplary CD40 single-chain antibody is as shown in amino acid residues 161-268 of SEQ ID NO: 1, an exemplary coding sequence thereof may be as shown in nucleotide residues 481-804 of SEQ ID NO: 2. The light chain variable region and the heavy chain variable region may be connected by a hinge region containing GS. An exemplary hinge region sequence may be as shown in amino acid residues 147-160 of SEQ ID NO: 1. In certain embodiments, the amino acid sequence of the CD40 single-chain antibody suitable herein is as shown in amino acid residues 21-268 of SEQ ID NO: 1, and its exemplary coding sequence may be as shown in nucleotide residues 60-804 of SEQ ID NO: 2.

[0087] Inhibitory antibodies useful in the present disclosure include, but are not limited to, immune checkpoint inhibitory antibodies known in the art, such as antibodies against one or more of the following antigens: PD-1, CTLA4, PDL1, PDL2, PDL3, TIM3, LAG3, CD47, BTLA, TIGIT, CD160, LAIR1, B7-H1, B7-1, VSIR and CD244. In certain embodiments, the inhibitory antibody used in the present disclosure is an antibody against PD-1 or CD47. In certain embodiments, the PD-1 antibody is a single chain antibody. The amino acid sequence of the light chain variable region (V_L region) of an exemplary PD-1 single chain antibody is as shown in amino acid residues 21-131 of SEQ ID NO: 3, an exemplary coding sequence thereof is as shown in nucleotide residues 60-393 of SEQ ID NO: 4; the amino acid sequence of the heavy chain variable region of an exemplary PD-1 single-chain antibody is as shown in amino acid residues 147-266 of SEQ ID NO: 3, an exemplary coding sequence thereof may be as shown in nucleotide residues 439-798 of SEQ ID NO: 4. The light chain variable region and the heavy chain variable region may be connected by a hinge region containing GS. An exemplary hinge region sequence may be as shown in amino acid residues 132-146 of SEQ ID NO: 3. In certain embodiments, the amino acid sequence of the PD-1 single-chain antibody suitable herein is as shown in amino acid residues 21-266 of SEQ ID NO: 3, and its exemplary coding sequence may be as shown in nucleotide residues 60-798 of SEQ ID NO: 4.

[0088] In certain embodiments, the antigen-binding sequence used herein is a ligand for proteins that function in the tumor microenvironment (e.g., related to the growth, migration and phagocytosis escaping of tumor cells, etc.), especially a ligand of proteins expressed on the surface of cancer cells, such as CD47. In certain embodiments, the amino acid sequence of the CD47 ligand is as shown in amino acid residues 21-138 of SEQ ID NO: 5, and its exemplary coding sequence may be as shown in nucleotide residues 60-414 of SEQ ID NO: 6.

3. Antibodies expressed by CAR-T cells

[0089] In the present disclosure, antibodies expressed by CAR-T cells usually contain the antigen-binding sequence described herein and the mutant Fc segment. The two can be directly connected, or can be connected by suitable linkers.

[0090] In certain embodiments, the antibodies of the disclosure contain optional signal peptides, agonistic or inhibitory antibodies, and mutant Fc segments described herein. For example, the antibody of the disclosure may contain an optional signal peptide, an antibody against an antigen selected from the following antigens, and the mutant Fc segments described herein: CD28, CD137, CD134, CD40, CD40L, ICOS, HVEM, CD2, CD27, CD30, GITR, LIGHT, DR3, SLAM, CD226, CD80 and CD86. Alternatively, the antibody of the present disclosure may contain an optional signal peptide, an antibody against an antigen selected from the following antigens, and the mutant Fc segments described herein: PD-1, CTLA4, PDL1, PDL2, PDL3, TIM3, LAG3, CD47, BTLA, TIGIT, CD160, LAIR1, B7-H1, B7-1, VSIR and CD244. Preferably, the antigen-binding sequence is a single-chain antibody formed by a light chain variable region and a heavy chain variable region of an antibody against the antigen, or the ligand of a protein expressed on the surface of a cancer cell.

[0091] In certain embodiments, the antibody further includes a signal peptide sequence. The signal peptide is a short peptide chain (5-30 amino acids in length) that guides the transfer of newly synthesized proteins to the secretory pathway, which often refers to the amino acid sequence at the N-terminus (sometimes not necessarily the N-terminus) of the newly synthesized polypeptide chains and used to guide the transmembrane transfer (location) of proteins, by directing proteins to subcellular organelles having different membrane structures. The signal peptide may be a secretory signal peptide or a membrane-bound signal peptide.

[0092] Any suitable signal peptide sequence can be used in the present disclosure. In certain embodiments, the signal peptide may be a CD8 signal peptide, a CD28 signal peptide, a CD4 signal peptide, or a light chain signal peptide. In certain embodiments, the signal peptide in the antibody of the present disclosure is the light chain signal peptide, and its exemplary amino acid sequence may be as shown in amino acid residues 1-20 of SEQ ID NO: 1, and an exemplary coding sequence may be as shown in nucleotide residues 1-60 of SEQ ID NO: 2. In certain embodiments, the signal peptide in the antibody of the present disclosure is the CD8 signal peptide, its exemplary amino acid sequence may be as shown in amino acid residues 1-21 of SEQ ID NO: 7, its exemplary coding sequence may be as shown in nucleotide residues 1-63 of SEQ ID NO: 8; or, its exemplary amino acid sequence may be as shown in amino acid residues 1-22 of SEQ ID NO: 9, its exemplary coding sequence may be as shown in nucleotide residues 1-66 of SEQ ID NO: 10.

[0093] In certain embodiments, the antibody of the disclosure is the CD40 antibody, which contains an optional signal peptide, a CD40 single chain antibody, and the mutant Fc segment described herein; preferably, the signal peptide is a light chain signal peptide; preferably, the amino acid sequence of the light chain variable region (VL region) of the CD40 single chain antibody is as shown in amino acid residues 21-146 of SEQ ID NO: 1, an exemplary coding sequence thereof is as shown in nucleotide residues 60-438 of SEQ ID NO: 2; preferably, the amino acid sequence of the heavy chain variable region (VH region) of the CD40 single-chain antibody is as shown in amino acid residues 161-268 of SEQ ID NO: 1, an exemplary coding sequence thereof may be as shown in nucleotide residues 481-804 of SEQ ID NO: 2. In certain embodiments, the amino acid sequence of the CD40 single-chain antibody is as shown in amino acid residues 21-268 of SEQ ID NO: 1, and its exemplary coding sequence may be as shown in nucleotide residues 60-804 of SEQ ID NO: 2. In certain embodiments, the amino acid sequence of the mutant Fc segment is as shown in amino acid residues

269-497 of SEQ ID NO: 1, and its exemplary coding sequence may be as shown in nucleotide residues 805-1491 of SEQ ID NO: 2. In certain embodiments, the amino acid sequence of the CD40 antibody of the present disclosure is as shown in amino acid residues 21-497 of SEQ ID NO: 1, or as shown in SEQ ID NO: 1, and its exemplary coding sequence is as shown in nucleotide residues 60-1491 of SEQ ID NO: 2, or is as shown in SEQ ID NO: 2.

[0094] In certain embodiments, the antibody of the disclosure is the PD-1 antibody, which contains an optional signal peptide, a PD-1 single chain antibody, and the mutant Fc segment described herein; preferably, the signal peptide is a light chain signal peptide; preferably, the amino acid sequence of the light chain variable region (VL region) of the PD-1 single chain antibody is as shown in amino acid residues 21-131 of SEQ ID NO: 3, an exemplary coding sequence thereof is as shown in nucleotide residues 60-393 of SEQ ID NO: 4; preferably, the amino acid sequence of the heavy chain variable region (VH region) of the PD-1 single-chain antibody is as shown in amino acid residues 147-266 of SEQ ID NO: 3, an exemplary coding sequence thereof may be as shown in nucleotide residues 439-798 of SEQ ID NO: 4. In certain embodiments, the amino acid sequence of the PD-1 single-chain antibody is as shown in amino acid residues 21-266 of SEQ ID NO: 3, and its exemplary coding sequence may be as shown in nucleotide residues 60-798 of SEQ ID NO: 4. In certain embodiments, the amino acid sequence of the mutant Fc segment is as shown in amino acid residues 267-495 of SEQ ID NO: 3, and its exemplary coding sequence may be as shown in nucleotide residues 799-1485 of SEQ ID NO: 2. In certain embodiments, the amino acid sequence of the PD-1 antibody of the present disclosure is as shown in amino acid residues 21-495 of SEQ ID NO: 3, or as shown in SEQ ID NO: 3, and its exemplary coding sequence is as shown in nucleotide residues 60-1485 of SEQ ID NO: 4, or is as shown in SEQ ID NO: 4.

[0095] In certain embodiments, the antibody of the disclosure is a CD47 antibody, which contains an optional signal peptide, the ligand sequence of CD47, and the mutant Fc segment described herein; preferably, the signal peptide is the light chain signal peptide; preferably, the amino acid sequence of the ligand is as shown in amino acid residues 21-138 of SEQ ID NO: 5, and its exemplary coding sequence is as shown in the nucleotide residues 60-414 of SEQ ID NO: 6. In certain embodiments, the amino acid sequence of the mutant Fc segment is as shown in amino acid residues 139-367 of SEQ ID NO: 5, and its exemplary coding sequence may be as shown in nucleotide residues 415-1101 of SEQ ID NO: 6. In certain embodiments, the amino acid sequence of the CD47 antibody of the present disclosure is as shown in amino acid residues 21-367 of SEQ ID NO: 5, or as shown in SEQ ID NO: 5, and its exemplary coding sequence is as shown in nucleotide residues 60-1104 of SEQ ID NO: 6, or is as shown in SEQ ID NO: 6.

4. Chimeric antigen receptor (CAR)

[0096] The present invention relates to any chimeric antigen receptor that can be expressed in T cells, including but not limited to those recognize, target, or specifically bind to one or more of the following antigens: CD19, CD20, CEA, GD2 (also known as B4GALNT1, β 1,4-acetyl-aminogalactosyltransferase 1), FR (Flavin reductase), PSMA (prostate specific membrane antigen), PMEL (premelanosome protein), CA9 (carbonic anhydrase IX), CD171/L1-CAM, IL-13R α 2, MART-1 (also known as mucin-A), ERBB2, NY-ESO-1 (also known as CTAG1B, cancer/testis antigen 1B), MAGE (melanoma-associated antigen E1) family proteins, BAGE (B melanoma antigen family) family proteins, GAGE (growth hormone releasing factor) family proteins, AFP (a-fetoprotein), MUC1 (mucin 1, cell surface related), CD22, CD23, CD30, CD33, CD44v7/8, CD70, VEGFR1, VEGFR2, IL-11R α , EGP-2, EGP-40, FBP, GD3 (also known as ST8SIA1, ST8 α -N-acetyl-ceramide α -2,8-sialic acid convertase 1), PSCA (prostate stem cell antigen), FSA (also known as KIAA1109), PSA (also known as KLK3, kallikrein-related peptidase 3), HMGA2, fetal acetylcholine receptor, LeY (also known as FUT3), EpCAM, MSLN (mesothelin), IGFR1, EGFR, EGFRvIII, ERBB3, ERBB4, CA125 (also known as MUC16, mucin 16, cell surface related), CA15-3, CA19-9, CA72-4, CA242, CA50, CYFRA21-1, SCC (also known as SERPINB3), AFU (also known as FUCA1), EBV-VCA, POA (also known as VDR, vitamin D (1,25-dihydrovitamin D3) receptor), β 2-MG (β -2-microglobulin) and PROGRP (GRP gastrin releasing peptide).

[0097] The chimeric antigen receptor of the present invention usually contains an optional signal peptide sequence, antigen recognition region, hinge region, transmembrane region, intracellular co-stimulatory signal domain and intracellular signal domain.

[0098] The signal peptides suitable for the chimeric antigen receptor herein may be as described above, and may be secretory signal peptides or membrane-bound signal peptides, including but not limited to CD8 signal peptide, CD28 signal peptide, CD4 signal peptide and a light chain signal peptide. In certain embodiments, the signal peptide in the chimeric antigen receptor of the present disclosure is a light chain signal peptide, and its exemplary amino acid sequence may be as shown in amino acid residues 1-20 of SEQ ID NO: 11, and an exemplary coding sequence may be as shown in nucleotide residues 1-60 of SEQ ID NO: 12. In certain embodiments, the signal peptide in the antibody of the present disclosure is the CD8 signal peptide, its exemplary amino acid sequence may be as shown in amino acid residues 1-21 of SEQ ID NO: 7, its exemplary coding sequence may be as shown in nucleotide residues 1-63 of SEQ ID NO: 8; or, its exemplary amino acid sequence may be as shown in amino acid residues 1-22 of SEQ ID NO: 9, its exemplary coding sequence may be as shown in nucleotide residues 1-66 of SEQ ID NO: 10.

[0099] The antigen recognition region may be a single chain antibody (scFv). The single chain antibody may be a

scFv commonly used in the art that recognizes a target antigen, including but not limited to the scFv formed by the light chain variable region and heavy chain variable region of an antibody that recognizes, targets, or specifically binds to one or more of the aforementioned antigens. In certain embodiments, the amino acid sequence of the present disclosure that recognizes the target antigen is a single chain antibody that recognizes, targets, or specifically binds to CD19, mesothelin, EGFR, or mucin. In certain embodiments, the antigen recognition region of the disclosure consists of amino acid sequences that target the ErbB receptor family.

[0100] For example, the amino acid sequence of the light chain variable region of an exemplary single-chain antibody that recognizes CD19 may be as shown in amino acid residues 22-128 of SEQ ID NO: 7, and its exemplary coding sequence may be as shown in nucleotide residues 64-384 of SEQ ID NO: 8. For example, the amino acid sequence of the heavy chain variable region of an exemplary single-chain antibody that recognizes CD19 may be as shown in amino acid residues 144-263 of SEQ ID NO: 7, and its exemplary coding sequence may be as shown in nucleotide residues 430-789 of SEQ ID NO: 8. In certain embodiments, the amino acid sequence of an exemplary single-chain antibody that recognizes CD19 is as shown in amino acid residues 22-263 of SEQ ID NO: 7, and its exemplary coding sequence may be as shown in nucleotide residues 64-789 of SEQ ID NO: 8.

[0101] An exemplary scFv that recognizes mesothelin antigen may be a single chain antibody well known in the art against mesothelin antigen. Preferably, the amino acid sequences of the light chain variable region and the heavy chain variable region of the single-chain antibody are derived from an antibody against the amino acid sequence of the membrane-proximal end of mesothelin. Preferably, the anti-mesothelin single chain antibody described herein is a single chain antibody against Region I or III of mesothelin. Preferably, the amino acid sequences of the light chain variable region and the heavy chain variable region of the single-chain antibody are derived from an antibody against the amino acid sequence of Region I or III of mesothelin. In some embodiments, the amino acid sequence of mesothelin Region I is as shown in SEQ ID NO: 21; the amino acid sequence of mesothelin Region III is as shown in SEQ ID NO: 22. The amino acid sequence of an exemplary anti-mesothelin Region I single-chain antibody is as shown in SEQ ID NO: 23. The amino acid sequence of the light chain variable region of an exemplary single chain antibody against mesothelin Region III is as shown in amino acid residues 23-146 of SEQ ID NO: 9, an exemplary coding sequence thereof is as shown in nucleotide residues 67-438 of SEQ ID NO: 10; the amino acid sequence of the heavy chain variable region of an exemplary single chain antibody against mesothelin Region III is as shown in amino acid residues 162-272 of SEQ ID NO: 9, an exemplary coding sequence thereof may be as shown in nucleotide residues 484-816 of SEQ ID NO: 10. In certain embodiments, the amino acid sequence of the scFv that recognizes mesothelin antigen is as shown in amino acid residues 23-272 of SEQ ID NO: 9, and its exemplary coding sequence may be as shown in nucleotide residues 67-816 of SEQ ID NO: 10. Herein, unless otherwise specified, mesothelin refers to the mesothelin fragment anchored on the membrane.

[0102] In the present disclosure, an exemplary antigen recognition region targeting the ErbB receptor family contains a fusion protein of the natural TIE and Herin. Herein, TIE is a chimeric polypeptide consisting of 7 amino acids at the N-terminus of human transcription growth factor α (TGF α) and 48 amino acids at the C-terminus of epidermal growth factor (EGF). Preferably, the amino acid sequence of the TIE is as shown in amino acid residues 23-77 of SEQ ID NO: 13, and its exemplary coding sequence may be as shown in nucleotide residues 67-231 of SEQ ID NO: 14. Herein, Herin is the 79 amino acids encoded by intron 8 in Herstatin. Preferably, the amino acid sequence of Herin is as shown in amino acid residues 93-171 of SEQ ID NO: 13. In the present disclosure, the codons encoding amino acids of Herin are optimized. Therefore, the preferred nucleotide sequence of Herin of the present disclosure is as shown in the nucleotide residues 277-513 of SEQ ID NO: 14. Generally, TIE and Herin can be connected by a rigid linker sequence. An example of a rigid linker sequence is two or more repeats of EAAAK, also referred to herein as the EAAAK linker. In certain embodiments, an exemplary rigid linker sequence is as shown in amino acid residues 78-92 of SEQ ID NO: 13, and its exemplary coding sequence may be as shown in nucleotide residues 232-276 of SEQ ID NO: 14. In certain embodiments, the antigen recognition region described herein is as shown in amino acid residues 23-171 of SEQ ID NO: 13, and its exemplary coding sequence may be as shown in nucleotide residues 67-513 of SEQ ID NO: 14.

[0103] In the present disclosure, an exemplary antigen recognizing region that recognizes mucin (Muc1) antigen may be a scFv of mucin, and the single chain antibody may be a single chain antibody well known in the art against Muc1 antigen. In certain embodiments, the amino acid sequences of the light chain variable region and the heavy chain variable region of the single chain antibody are derived from an antibody against the amino acid sequence of the membrane-proximal end of Muc1. In certain embodiments, the amino acid sequence of the membrane-proximal end of Muc1 is as shown in SEQ ID NO: 24. The amino acid sequence of the light chain variable region of an exemplary anti-Muc1 single-chain antibody may be as shown in amino acid residues 23-133 of SEQ ID NO: 15, and its exemplary coding sequence may be as shown in nucleotide residues 67-399 of SEQ ID NO: 16. The amino acid sequence of the heavy chain variable region of an exemplary anti-Muc1 single-chain antibody may be as shown in amino acid residues 149-269 of SEQ ID NO: 15, and its exemplary coding sequence may be as shown in nucleotide residues 445-809 of SEQ ID NO: 16. In certain embodiments, the amino acid sequence of an exemplary single-chain antibody that recognizes mucin antigen is as shown in amino acid residues 23-269 of SEQ ID NO: 15, and its exemplary coding sequence may be as shown in

nucleotide residues 67-807 of SEQ ID NO: 16.

[0104] Herein, the antigen recognition region that recognizes EGFR may be a single chain antibody formed by the light chain variable region and the heavy chain variable region of an antibody specific for EGFR, and the single chain antibody may be a single chain antibody well known in the art against EGFR. The amino acid sequence of the light chain variable region of an exemplary single-chain antibody that recognizes EGFR may be as shown in amino acid residues 23-129 of SEQ ID NO: 17, and its exemplary coding sequence may be as shown in nucleotide residues 67-387 of SEQ ID NO: 18. The amino acid sequence of the heavy chain variable region of an exemplary single-chain antibody that recognizes EGFR may be as shown in amino acid residues 145-263 of SEQ ID NO: 17, and its exemplary coding sequence may be as shown in nucleotide residues 433-789 of SEQ ID NO: 18. In certain embodiments, the amino acid sequence of an exemplary single-chain antibody that recognizes EGFR is as shown in amino acid residues 23-263 of SEQ ID NO: 17, and its exemplary coding sequence may be as shown in nucleotide residues 67-789 of SEQ ID NO: 18.

[0105] In this article, the hinge region refers to the region between the CH1 and CH2 functional regions of the immunoglobulin heavy chain. The hinge region is rich in proline, does not form an alpha helix, and is prone to stretching and distortion to a certain degree, which facilitates the complementary binding between the antigen binding site of the antibody and the epitope. The hinge region suitable herein may be selected from any one or more of the group consisting of an extracellular hinge region of CD8, an IgG1 Fc CH2CH3 hinge region, a IgD hinge region, a extracellular hinge region of CD28, a IgG4 Fc CH2CH3 hinge region and a extracellular hinge region of CD4. The hinge region is preferably a hinge region having 50 or more amino acid residues in length, more preferably 80 or more amino acid residues in length. In certain embodiments, the CD8 α hinge region or IgG4 Fc CH2CH3 hinge region is used herein. In certain embodiments, the amino acid sequence of the CD8 α hinge region is as shown in amino acid residues 264-308 of SEQ ID NO: 7, and its exemplary coding sequence is shown in nucleotide residues 790-924 of SEQ ID NO: 8; in other embodiments, the amino acid sequence of CD8 α hinge region is as shown in amino acid residues 264-318 of SEQ ID NO: 17, and its exemplary coding sequence is as shown in nucleotide residues 790-954 of SEQ ID NO: 18. The amino acid sequence of an exemplary IgG4 CH2CH3 hinge region is as shown in amino acid residues 273-500 of SEQ ID NO: 9, and its exemplary coding sequence may be as shown in nucleotide residues 817-1500 of SEQ ID NO: 10.

[0106] The transmembrane region may be one of the CD28 transmembrane region, the CD8 transmembrane region, the CD3 ζ transmembrane region, the CD134 transmembrane region, the CD137 transmembrane region, the ICOS transmembrane region, and the DAP10 transmembrane region. In certain embodiments, the transmembrane region is the CD8 transmembrane region, and its exemplary amino acid sequence is as shown in amino acid residues 309-332 of SEQ ID NO: 7, and its exemplary coding sequence is as shown in nucleotide residues 925-996 of SEQ ID NO: 8. In certain embodiments, the amino acid sequence of the CD8 transmembrane region is as shown in amino acid residues 319-344 of SEQ ID NO: 17, and its exemplary coding sequence may be as shown in nucleotide residues 955-1032 of SEQ ID NO: 18. In certain embodiments, the transmembrane region is the CD28 transmembrane region, and its amino acid sequence is as shown in amino acid residues 501-528 of SEQ ID NO: 9, and its coding sequence is as shown in nucleotide residues 1501-1584 of SEQ ID NO: 10.

[0107] The intracellular co-stimulatory signal domain includes the intracellular domain of the co-stimulatory signal molecule, which can be selected from the group consisting of a intracellular domains of CD28, a CD134/OX40, CD137/4-1BB, a lymphocyte-specific protein tyrosine kinase (LCK), a inducible T cell co-stimulatory factor (ICOS) and a DNAX activating protein 10 (DAP10). In certain embodiments, the intracellular domain of the co-stimulatory signaling molecule is the intracellular domain of CD137/4-1BB; preferably, the amino acid sequence of the CD137/4-1BB is as shown in the amino acid residues 333-374 of SEQ ID NO: 7, and its exemplary coding sequence is as shown in the nucleotide residues 997-1122 of SEQ ID NO: 8. In certain embodiments, the intracellular co-stimulatory signal domain is the CD28 intracellular region, and its exemplary amino acid sequence is as shown in amino acid residues 529-569 of SEQ ID NO: 9, and its exemplary coding sequence is as shown in nucleotide residues 1585-1707 of SEQ ID NO: 10.

[0108] The intracellular signal domain is preferably an immunoreceptor tyrosine activation motif, which may be the CD3 ζ intracellular signal domain or Fc ϵ R1 γ intracellular signal domain; preferably is the CD3 ζ intracellular signal domain, preferably the amino acid sequence of the CD3 ζ intracellular signal domain is as shown in the amino acid residues 375-486 of SEQ ID NO: 7, its exemplary coding sequence may be as shown in the nucleotide residues 1123-1458 of SEQ ID NO: 8.

[0109] In certain embodiments, the chimeric antigen receptor herein contains, in the order from the N-terminus to the C-terminus, an optional signal peptide sequence, antigen recognition region, CD8 α hinge region or IgG4 CH2CH3 hinge region, CD8 transmembrane region or CD28 transmembrane region, 4-1BB or CD28 intracellular domain and CD3 ζ intracellular signal domain. Preferably, the chimeric antigen receptor of the present disclosure is: a chimeric antigen receptor targeting CD19, with the amino acid sequence thereof is as shown in amino acid residues 22-486 of SEQ ID NO: 7, or as shown in SEQ ID NO: 7, and the exemplary coding sequence thereof is as shown in nucleotide residues 64-1458 of SEQ ID NO: 8, or as shown in SEQ ID NO: 8; a chimeric antigen receptor targeting mesothelin, with the amino acid sequence thereof is as shown in amino acid residues 23-681 of SEQ ID NO: 9, or as shown in SEQ ID NO: 9, the exemplary coding sequence thereof is as shown in nucleotide residues 67-2043 of SEQ ID NO: 10, or as shown in SEQ

ID NO: 10, or the amino acid sequence thereof is as shown in amino acid residues 21-679 of SEQ ID NO: 11, or as shown in SEQ ID NO: 11, the exemplary coding sequence thereof is as shown in nucleotide residues 61-2037 of SEQ ID NO: 12, or as shown in SEQ ID NO: 12; an antigen recognition region targeting ErbB family, with the amino acid sequence thereof is as shown in amino acid residues 23-580 of SEQ ID NO: 13, or as shown in SEQ ID NO: 13, and the exemplary coding sequence thereof is as shown in nucleotide residues 67-1740 of SEQ ID NO: 14, or as shown in SEQ ID NO: 14; a chimeric antigen receptor targeting mucin, with the amino acid sequence thereof is as shown in amino acid residues 23-678 of SEQ ID NO: 15, or as shown in SEQ ID NO: 15, and the exemplary coding sequence thereof is as shown in nucleotide residues 67-2034 of SEQ ID NO: 16, or as shown in SEQ ID NO: 16; or a chimeric antigen receptor targeting EGFR, with the amino acid sequence thereof is as shown in amino acid residues 23-497 of SEQ ID NO: 17, or as shown in SEQ ID NO: 17, and the exemplary coding sequence thereof is as shown in nucleotide residues 67-1491 of SEQ ID NO: 18, or as shown in SEQ ID NO: 18.

[0110] The above elements forming the chimeric antigen receptor herein, such as the signal peptide, the light chain variable region and heavy chain variable region of the single chain antibody against Mucl, the hinge region, the transmembrane region, the intracellular co-stimulatory signal domain and the intracellular signal domains, etc., can be directly connected to each other, or can be connected by linker sequences. The linker sequence may be a linker sequence known in the art suitable for antibodies, for example, a linker sequence containing G and S. The linker may be 3-25 amino acid residues in length, for example, 3-15, 5-15, 10-20 amino acid residues. In certain embodiments, the linker sequence is a polyglycine linker sequence. The number of glycine in the linker sequence is not particularly limited, but is usually 2-20, such as 2-15, 2-10, 2-8. In addition to glycine and serine, the linker can also contain other known amino acid residues, such as alanine (A), leucine (L), threonine (T), glutamic acid (E), phenylalanine (F), arginine (R), glutamine (Q), etc.

5. Nucleic acid sequence, nucleic acid construct and vector and preparation method thereof

[0111] The invention also includes a nucleic acid sequence, selected from the group consisting of the coding sequences of the antibodies described herein or complementary sequences thereof. The nucleic acid sequence may be in the form of DNA or RNA. DNA can be single-stranded or double-stranded.

[0112] More specifically, the present disclosure includes the coding sequence of an antibody containing an optional signal peptide, the antigen binding sequence described herein, and the mutant Fc segment, or a complementary sequence thereof. Exemplary coding sequences include but are not limited to the coding sequence as shown in SEQ ID NO: 2 or its nucleotide residues 60-1491; the coding sequence as shown in SEQ ID NO: 4 or its nucleotide residues 60-1485; and the coding sequence as shown in SEQ ID NO: 6 or its nucleotide residues 60-1104.

[0113] The disclosure also includes the coding sequences of chimeric antigen receptors described herein, or complementary sequences thereof. The coding sequence of an exemplary chimeric antigen receptor includes the coding sequence that codes the chimeric antigen receptor containing, in the order from the N-terminus to the C-terminus, an optional signal peptide sequence, antigen recognition region, CD8 α hinge region or IgG4 CH2CH3 hinge region, CD8 transmembrane region or CD28 transmembrane region, the intracellular domain of 4-1BB or CD28 and the intracellular signal domain of CD3 ζ . Exemplary coding sequences include the coding sequence as shown in SEQ ID NO: 8 or its nucleotide residues 64-1458, the coding sequence as shown in SEQ ID NO: 10 or its nucleotide residues 67-2043, the coding sequence as shown in SEQ ID NO: 12 or its nucleotide residues 67-2037, the coding sequence as shown in SEQ ID NO: 14 or its nucleotide residues 67-1740, the coding sequence as shown in SEQ ID NO: 16 or its nucleotide residues 67-2034, and the coding sequence as shown in SEQ ID NO: 18 or its nucleotide residues 67-1491.

[0114] The present disclosure also includes nucleic acid constructs containing the coding sequence of the antibody or chimeric antigen receptor of the present disclosure, or a complementary sequence thereof.

[0115] In certain embodiments, the nucleic acid construct is an expression cassette that contains operably linked: a promoter sequence, the coding sequence of an antibody or chimeric antigen receptor or complementary sequence thereof, and optionally a polyA tailing signal sequence. The promoter sequence is usually operably linked to the coding sequence described herein. The promoter can be any nucleotide sequence having transcriptional activity in the selected host cell, including mutant, truncated, and hybrid promoters, which can be obtained from a gene encoding an extracellular or intracellular polypeptide that is homologous or heterologous to the host cell. The expression cassette usually contains a transcription terminator sequence, which is recognized by the host cell to terminate transcription. The terminator sequence is operably linked to the 3' end of the coding sequence described herein. Any terminator that is functional in the selected host cell may be used in the present disclosure.

[0116] In certain embodiments, the nucleic acid construct is a vector. Different vectors include but are not limited to plasmids, phagemids, phage derivatives, animal viruses and cosmids. The vector may be an expression vector, preferably a eukaryotic expression vector. The expression vector can be provided to the cell as a viral vector. Viruses that can be used as vectors include, but are not limited to retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. The vector may also be an integration vector for integrating the coding sequence or its complementary

sequence into the host cell.

[0117] Generally, suitable vectors contain a replication origin that functions in at least one organism, promoter sequences, convenient restriction enzyme sites, and one or more selectable markers. For example, in certain embodiments, the present disclosure uses a retroviral vector that contains a replication initiation site, 3'LTR, 5'LTR, the coding sequence of the antibody or chimeric antigen receptor described herein, and optional selectable markers.

[0118] Here, suitable promoters include, but are not limited to, the immediate early cytomegalovirus (CMV) promoter sequence. Such promoter sequence is a strong constitutive promoter sequence capable of driving high-level expression of any polynucleotide sequence operably linked thereto. Another example of a suitable promoter is elongation growth factor-1 α (EF-1 α). However, other constitutive promoter sequences can also be used, including but not limited to simian virus 40 (SV40) early promoter, mouse breast cancer virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, avian leukemia virus promoter, EB virus immediate early promoter, Ruth's sarcoma virus promoter, and human gene promoters, such as but not limited to actin promoter, myosin promoter, heme promoter and creatine kinase promoter. Furthermore, inducible promoters may also be considered. The use of an inducible promoter provides a molecular switch that can turn on the expression of a polynucleotide sequence operably linked to the inducible promoter when desired, and turn off the expression when expression is undesirable. Examples of inducible promoters include, but are not limited to, metallothionein promoter, glucocorticoid promoter, progesterone promoter, and tetracycline promoter.

[0119] In some embodiments, various promoter sequences published in CN201510021408.1 can be used herein, including but not limited to: CCEF promoter as shown in SEQ ID NO: 5 of the application, comprising mCMV enhancer, hCMV enhancer, and EF1 α promoter; TCEF promoter as shown in SEQ ID NO: 7, comprising CD3e enhancer, mCMV enhancer, hCMV enhancer and EF1 α promoter; CCEFI promoter as shown in SEQ ID NO: 8, comprising mCMV enhancer, hCMV enhancer and intron-containing EF1 α promoter; TEFI promoter as shown in SEQ ID NO: 3, comprising CD3e enhancer and intron-containing EF1 α promoter; and TCEFI promoter as shown in SEQ ID NO: 3, comprising CD3e enhancer, mCMV enhancer, hCMV enhancer and intron-containing EF1 α promoter. The entire contents of this application are incorporated herein by reference.

[0120] Selectable markers include either or both of selectable marker genes and reporter genes, which facilitate identification and selection of the expressing cells from a population of cells infected with viral vectors. Useful selectable marker genes include, for example, antibiotic resistance genes, such as neo and the like. Suitable reporter genes may include genes encoding luciferase, β -galactosidase, chloramphenicol acetyltransferase, secreted alkaline phosphatase, or green fluorescent protein genes.

[0121] In certain embodiments, the coding sequence of the chimeric antigen receptor and the coding sequence of the antibody described herein can be cloned separately into vectors (also called integration vectors), especially transposition vectors, for integrating the nucleic acid sequences of interest into the genome of the host cell. In certain embodiments, the transposition vector is a eukaryotic expression vector containing a transposition element selected from the group consisting of piggybac, sleeping beauty, frog prince, Tn5, or Ty. Such transposition vectors contain the 5' inverted terminal repeat (5'LTR) of the corresponding transposon and the 3' inverted terminal repeat (3'LTR) of the corresponding transposon. The transposase may be a transposase from a piggybac, sleeping beauty, frog prince, Tn5 or Ty transposition system. If a transposase from a different transposition system is used, the sequences of 5'LTR and 3'LTR in the vector are also changed accordingly into sequences suitable for the transposition system, which can be easily determined by those skilled in the art. In certain embodiments, the expression cassette of the CAR or antibody of the present disclosure is located between 5'LTR and 3'LTR, the expression cassette including the corresponding promoter sequence, the coding sequence of the CAR or antibody, and a polyA tailed signal sequence.

[0122] In certain embodiments, the transposase is a transposase from the piggybac transposition system. Therefore, in these embodiments, the 5' inverted terminal repeat sequence and the 3' inverted terminal repeat sequence of the transposon are the 5' inverted terminal repeat sequence and the 3' inverted terminal repeat sequence of the piggybac transposon, respectively. In certain embodiments, the transposon 5' inverted terminal repeat sequence is shown in SEQ ID NO: 1 of CN201510638974.7 (the contents of which are incorporated herein by reference). In certain embodiments, the 3' inverted terminal repeat sequence of the transposon is shown in SEQ ID NO: 4 of CN 201510638974.7. In certain embodiments, the piggybac transposase is a transposase containing c-myc nuclear localization signal coding sequence. In certain embodiments, the coding sequence of piggybac transposase is shown in SEQ ID NO: 5 of CN201510638974.7.

[0123] The promoter of the transposase coding sequence may be various promoters known in the art for controlling the expression of the transposase coding sequence. In certain embodiments, the CMV promoter is used to control the expression of the transposase coding sequence. The sequence of the CMV promoter may be as shown in SEQ ID NO: 6 of CN 201510638974.7.

[0124] In certain embodiments, the vector herein containing the coding sequence of the chimeric antigen receptor is the pNB328 vector disclosed in CN201510638974.7. The coding sequence of the chimeric antigen receptor of the present disclosure can be prepared and cloned into a suitable vector by conventional methods in the art

[0125] In certain embodiments, the vector used to integrate the gene of interest into the genome of the host cell does

not contain a transposase coding sequence. For example, such vectors can be obtained by removing the transposase coding sequence from the pNB328 vector. Generally, such vectors can be used to integrate the expression cassette of the antibody of the present disclosure into the genome of the host cell.

[0126] The nucleic acid sequences described herein can be obtained by methods well known in the art, such as PCR amplification. For example, sequences of interest can be amplified by use of primers designed based on the nucleotide sequences disclosed herein, and commercially available cDNA libraries or cDNA libraries prepared according to conventional methods known to those skilled in the art as templates. When the sequence is long, two or more PCR amplifications may be necessary, and then the amplified fragments from amplifications are spliced together in the correct order.

[0127] It should be understood that it is often necessary to add appropriate restriction site(s) during the gene cloning process, which will inevitably introduce one or more irrelevant residues at the end(s) of the expressed amino acid sequence(s), while not affect the activity of the obtained sequence. In order to construct the fusion protein, promote the expression of the recombinant protein, obtain the recombinant proteins automatically secreted by the host cells, or facilitate the purification of the recombinant proteins, it is often necessary to add amino acid(s) to the N-terminus, C-terminus, or within other suitable regions of the recombinant protein, and the added amino acid(s) include but are not limited to suitable linker peptides, signal peptides, leader peptides, terminally extended amino acid(s), etc. Therefore, the N-terminus or C-terminus of the CAR herein may further contains one or more polypeptide fragments as protein labels. Any suitable label can be used for this disclosure. For example, the labels may be FLAG, HA, HA1, c-Myc, Poly-His, Poly-Arg, Strep-TagII, AU1, EE, T7, 4A6, ϵ , B, gE, and Ty1. These labels can be used to purify proteins.

6. Host cells and preparation thereof

[0128] The present disclosure also provides a host cell containing the nucleic acid construct described herein, or expressing the antibody and/or chimeric antigen receptor described herein.

[0129] The host cell of the present disclosure may be various cells well known in the art suitable for expressing antibodies, such as 293 or CHO cells. Such host cells may contain expression vectors that express the antibodies described herein.

[0130] In certain embodiments, the host cell of the present disclosure is a cell for simultaneously expressing the antibody and the chimeric antigen receptor described herein, which contains the coding sequence or expression cassette of the antibody and the chimeric antigen receptor described herein; preferably, two expression cassettes are integrated into the genome of the cell, i.e., the expression cassette of the antibody and the expression cassette of the chimeric antigen receptor, as described herein. Preferably, such cells are T cells. T cells of interest include but are not limited to peripheral blood T lymphocytes, cytotoxic T lymphocytes (CTL), helper T cells, inhibitory/regulatory T cells, $\gamma\delta$ T cells, and cytokine-induced killer cells (CIK), tumor infiltration lymphocytes (TIL), or T cells of a mixed cell population.

[0131] Preferably, the T cell is transfected with a vector for integration of an expression cassette of the chimeric antigen receptor into its genome and containing a transposase coding sequence, and a vector for integration of an expression cassette of the antibody as described herein into its genome and not containing a transposase coding sequence. More preferably, the T cells are transfected with a vector containing an expression cassette of the chimeric antigen receptor constructed based on the pNB328 vector and a vector containing an expression cassette of the antibody described herein constructed based on the pS328 vector (comprising no transposase coding sequence compared to pNB328). In certain embodiments, the vector for integration of an expression cassette of the chimeric antigen receptor into the T cell genome and containing a transposase coding sequence comprises sequentially a 5'LTR, a promoter, the coding sequence of the CD8 signal peptide or a light chain signal peptide, the coding sequence of the antigen recognition region, the coding sequence of the CD8 α hinge region or the IgG4 CH2CH3 hinge region, the coding sequence of the CD8 or CD28 transmembrane region, the coding sequence of the 4-1BB or CD28 intracellular domain, the coding sequence of the CD3 ζ intracellular signal domain, a polyA tailing signal sequence, a 3'LTR and the coding sequence of a transposase and its promoter; the vector for integration of an expression cassette of the antibody as described herein into the T cell genome and not containing a transposase coding sequence comprises sequentially between the 5'LTR and the 3'LTR a promoter, the coding sequence of the light chain signal peptide or the CD8 signal peptide, the coding sequence of the antibody, and a polyA tailing signal sequence. Preferably, the antigen recognition region is an antigen recognition region targeting CD19, mesothelin, mucin, EGFR or ErbB family, preferably their amino acids and coding sequences are as described above. Preferably, the antibody is an anti-PD-1 antibody, an anti-CD47 antibody or an anti-CD40 antibody; preferably, their amino acid sequences and coding sequences are as described above.

[0132] Conventional transfection methods can be used to transfer the vectors of the present disclosure into cells of interest, including but not limited to: viral transduction, microinjection, particle bombardment, gene gun transformation, and electroporation. In certain embodiments, the vectors described herein are transfected into cells of interest by electroporation. Preferably, during transfection, the mass ratio of the vector containing the coding sequence of the chimeric antigen receptor to the vector containing the coding sequence of the antibody is 1-7:1-7, such as 1-5:1-5, preferably 1-3:1-3, more preferably 1-2:1-2, more preferably 1-2:1.

7. Compositions and kits

[0133] The disclosure also provides a composition comprising the vector described herein, preferably the vector expressing the chimeric antigen receptor described herein and the vector expressing the antibody described herein. The composition is used at least to provide a vector for transfection.

[0134] In the composition of the present disclosure, the mass ratio of the vector expressing the chimeric antigen receptor described herein to the vector expressing the antibody described herein may be 1-7:1-7, such as 1-5:1-5, preferably 1-3:1-3, more preferably 1-2:1-2, more preferably 1-2:1. The composition may also contain suitable reagents, including but not limited to reagents for transfection.

[0135] The present disclosure also provides a kit containing a vector expressing the chimeric antigen receptor described herein and a vector expressing the antibody described herein, or a composition described herein. The kit may also be equipped with reagents and/or instruments for transferring the vector into cells.

[0136] The composition herein may be a pharmaceutical composition containing the T cells described herein or the T cells and the antibodies described herein expressed by the T cells. The pharmaceutical composition may contain a suitable pharmaceutically acceptable carrier or adjuvant. Suitable carriers or adjuvants include but are not limited to buffers and osmotic pressure regulators and the like. The pharmaceutical composition contains a therapeutically or prophylactically effective amount of T cells. The therapeutically or prophylactically effective amount of T cells can be determined according to factors such as the patient's condition.

8. Method and use

[0137] The disclosure also provides the use of the antibodies, their coding sequences or complementary sequences thereof, nucleic acid constructs, and host cells, as described herein, in the preparation of a medicament for treatment or prevention of malignant tumors. The tumor includes, but is not limited to, tumors associated with the antigen to which the antibody specifically binds to. The disclosure also includes the antibodies, their coding sequences or complementary sequences thereof, nucleic acid constructs, and host cells, as described herein, for treatment or prevention of malignant tumors.

[0138] The present disclosure also provides the use of the T cells described herein or the T cells and the antibodies expressed by the cells or pharmaceutical compositions thereof in the preparation of a medicament for treatment or prevention of malignant tumors. The present disclosure also includes the T cells described herein or the T cells and the antibodies expressed by the cells or pharmaceutical compositions thereof for treatment or prevention of malignant tumors.

[0139] The present disclosure also provides a method for treating or preventing malignant tumors, the method comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of the T cells or pharmaceutical composition thereof described herein.

[0140] The malignant tumor (cancer) that can be treated or prevented by the T cell of the present disclosure or the T cell and the antibody expressed by the T cell or a pharmaceutical composition thereof or the method described by the present disclosure may be the malignant tumor that can be treated or prevented by the antibody and/or chimeric antigen receptor expressed by the T cell. For example, when the chimeric antigen receptor is a chimeric antigen receptor targeting CD19, the malignant tumor may be malignant B-cell lymphoma, including acute B-lymphocytic leukemia (B-ALL), chronic B-lymphocytic leukemia (B-CLL), mantle cell lymphoma (MCL), non-Hodgkin lymphoma (NHL) and multiple myeloma (MM). When the chimeric antigen receptor targets mesothelin, the malignant tumor may be a cancer that abnormally expresses mesothelin on the surface of the cancer cell; preferably is adenocarcinoma, mesothelioma, lung cancer, colon cancer, colorectal cancer, breast cancer, ovarian cancer, cervical cancer, gastric cancer, bile duct cancer, gallbladder cancer, esophageal cancer, melanoma, non-small cell lung cancer, renal cell cancer, head and neck squamous cell carcinoma, rectal cancer, Hodgkin lymphoma, pancreatic cancer or prostate cancer; more preferably, the cancer is a cancer in which mesothelin and CA125/MUC16 are simultaneously highly expressed. When the chimeric antigen receptor targets the ErbB family, the malignant tumor may be a cancer that abnormally expresses at least one protein of EGFR family on the surface of the cancer cells, such as liver cancer, adenocarcinoma, lung cancer, colon cancer, colorectal cancer, breast cancer, ovarian cancer, cervical cancer, gastric cancer, bile duct cancer, non-small cell cancer, gallbladder cancer, esophageal cancer, melanoma, pancreatic cancer, urothelial cancer, head and neck cancer or prostate cancer. When the chimeric antigen receptor targets mucin, the malignant tumor may be a cancer that abnormally expresses mucin antigen on the surface of the cancer cells, such as liver cancer, adenocarcinoma, lung cancer, colon cancer, colorectal cancer, breast cancer, ovarian cancer, cervical cancer, gastric cancer, bile duct cancer, non-small cell cancer, gallbladder cancer, esophageal cancer, melanoma, pancreatic cancer, urothelial cancer, head and neck cancer or prostate cancer. When the chimeric antigen receptor targets EGFR, the malignant tumor may be a cancer that abnormally expresses EGFR on the surface of the cancer cells, such as glioblastoma, renal cancer, adenocarcinoma, lung cancer, colon cancer, colorectal cancer, breast cancer, ovarian cancer, cervical cancer, gastric cancer, bile duct cancer, gallbladder cancer, esophageal cancer, pancreatic cancer or prostate cancer. When the antibody is a CD40 antibody, a

suitable malignant tumor is a malignant tumor mediated by CD40, including but not limited to non-small cell carcinoma, melanoma, urothelial carcinoma, high frequency microsatellite instability (MSI-H) and head and neck cancer; when the antibody is a PD-1 antibody, a suitable malignant tumor is a malignant tumor mediated by PD-1, including but not limited to melanoma, colon cancer, prostate cancer, non-small cell lung cancer and renal cell carcinoma and other solid tumors.

When the antibody is a CD47 antibody, a suitable malignant tumor includes but is not limited to any tumor that expresses CD47 on the surface of the cancer cells.

[0141] The embodiments of the present disclosure will be illustrated by way of specific examples below. Those skilled in the art will understand that these examples are merely exemplary and should not be considered as limiting the scope of the present disclosure. The experimental methods without specifying the specific technology or conditions in the following examples generally used the conventional technology or conditions, such as those described in J. Sambrook et al., Molecular Cloning: A Laboratory Manual (3rd ed.), translated by Huang Peitang et al., Science Press, or followed the manufacturer's recommendation.

[0142] The used reagents or instruments without specifying the manufacturer are all conventional products that are commercially available.

Example 1: Construction of recombinant plasmid

[0143] Foreign genes (antibody or CAR) shown in Table 1 below were synthesized by Shanghai Generay Biotech Co., Ltd, with multiple-cloning restriction sites (BglII-XbaI-EcoRI-BamHI) introduced upstream and restriction sites (Sall-NheI-HindIII-SpeI) introduced downstream of the genes. The genes were inserted into pNB328 vectors or pS328 vectors (for the structure and sequence of pNB328, please refer to CN201510638974.7, the entire contents of which are incorporated herein by reference; compared to pNB328, pS328 lacks the coding sequence of the transposase; the chimeric antigen receptor genes were inserted into pNB328 vectors, and the antibody sequences were loaded into pS328 vectors) that were double digested by EcoRI+Sall to construct the recombinant plasmids.

Table 1

Foreign Gene Name	Foreign Gene Structure	Sequence No.	Recombinant Plasmid Name
mutant CD40 antibody	light chain signal peptide-CD40 scFv-mutant IgG4Fc	2	pS328- α CD40
mutant PD-1 antibody	light chain signal peptide-PD-1 scFv-mutant IgG4Fc	4	pS328-m279v or pS328-antiPD1
mutant CD47 antibody	light chain signal peptide-CD47 ligand-IgG4Fc	6	pS328- α CD47
CD19CAR	CD8 signal peptide-anti-CD 19 scFv-CD8 α hinge region-CD8 transmembrane region-4-IBB intracellular domain-CD3 ζ	8	pNB328-CD19CAR
mesoCAR	CD8 signal peptide-anti-meso scFv-mIgG4 Fc CH2CH3 hinge region-CD8 transmembrane region-CD28 intracellular domain-CD3 ζ	10	pNB328-mesoCAR
Meso3CAR	light chain signal peptide-anti-meso scFv-mIgG4 Fc CH2CH3 hinge region-CD8 transmembrane region-CD28 intracellular domain-CD3 ζ	12	pNB328-meso3CAR
ErbBCAR	CD8 signal peptide-T1E-EK linker-Her1n-mIgG4 Fc CH2CH3 hinge region-CD28 transmembrane region-CD28 intracellular domain-CD3 ζ	14	pNB328-EHCAR-EK-28TIZ
Muc 1CAR	CD8 signal peptide-anti-Muc1 scFv-mIgG4 Fc CH2CH3 hinge region-CD28 transmembrane region-CD28 intracellular domain-CD3 ζ	16	pNB328-Muc1CAR
EGFR-CAR	CD8 signal peptide-anti-EGFR scFv-mIgG4 Fc CH2CH3 hinge region-CD28 transmembrane region-CD28 intracellular domain-CD3 ζ	18	pNB328-EGFR-CAR

(continued)

Foreign Gene Name	Foreign Gene Structure	Sequence No.	Recombinant Plasmid Name
CD19CAR-2A- α CD40	CD19CAR-2A-mutant CD40 antibody	19*	pNB328-CD19CAR-2A- α CD40
α CD40-IRES-CD19CAR	mutant CD40 antibody-IRES-CD19CAR	20*	pNB328- α CD40-IRES-CD19CAR
mesoCAR-2A- α CD40	mesoCAR-2A-mutant CD40 antibody	19*	pNB328-mesoCAR-2A- α CD40
α CD40-IRES-mesoCAR	mutant CD40 antibody-IRES-mesoCAR	20*	pNB328- α CD40-IRES-mesoCAR
CD19CAR-2A-m279v	CD19CAR-2A-mutantPD-1 antibody	19*	pNB328-CD19CAR-2A-m279V
m279v-IRES-CD19C AR	mutant PD-1 antibody-IRES-CD19CAR	20*	pNB328-m279V-IRES-CD19CAR
mesoCAR-2A-antiPD11	mesoCAR-2A-mutant PD-1 antibody	19*	pNB328-mesoCAR-2A-antiPD1
antiPD1-IRES-mesoCAR	mutant PD-1 antibody-IRES -meso CAR	20*	pNB328-antiPD1-IRES-mesoCAR
WT CD40 antibody	light chain signal peptide-CD40 scFv-WT IgG4Fc	**	pS328- α CD40-wt
WT PD-1 antibody	light chain signal peptide-PD-1 scFv-WT IgG4Fc		pS328-m279v-wt
WT CD47 antibody	light chain signal peptide-CD47 ligand-WT IgG4Fc		pS328- α CD47-wt
*: SEQ ID NO: 19 shows the nucleotide sequence of 2A, SEQ ID NO: 20 shows the nucleotide sequence of IRES; the sequence of the rest of the foreign gene sequence is identical with the sequence of the foreign gene having the same name in the Table;			
**: the sequence of WT IgG4Fc is shown as SEQ ID NO: 25, which is identical with that of the mutant IgG4Fc except for L17E (CTG to GAG) and N79Q (AAC to CAG) mutations in the mutant.			

Example 2: Construction of CAR-T cells

[0144] Peripheral blood mononuclear cells (PBMCs) were isolated from patients' blood by Ficoll separation method. PBMCs were adherently cultured for 2-4h, and the non-adherent suspended cells were the initial T cells, which were collected in a 15ml centrifuge tube and centrifuged at 1200rpm for 3min, then the supernatant was discarded and saline was added. This step was repeated.

[0145] 5×10^6 cells were added in a 1.5ml centrifuge tube, and centrifuged at 1200rpm for 3min. The supernatant was discarded, a total of 100ul of the electroporation reagent of the Electroporation Kit (Lonza) was added, and then different recombinant plasmids were added according to Table 2 below. The cells were resuspended and mixed separately; the mixture was transferred to an electroporation cup, the cup was put into the electroporation instrument, the required program was selected, and electrical pulse was conducted; the micro pipette in the kit was used to transfer the electroporated cell suspension to a six-well plate supplemented with the medium (AIM-V medium containing 2% FBS), the cells were mixed well and cultured in a 37°C, 5% CO₂ incubator; after six hours, stimulating factor IL-2, anti-CD28 antibody and corresponding antigen (CD19, mesothelin, EGFR or mucin) or anti-CD3 antibody was added, and the cells were culture at 37°C, 5% CO₂ for 3 to 4 days to obtain corresponding T cells.

[0146] When two kinds of recombinant plasmids were transferred, 4ug of each recombinant plasmid was used; when one kind of recombinant plasmid was transferred, 6ug is used.

Table 2

Recombinant Plasmid	Name of Recombinant Cell
pNB328 empty vector	Mock T cells or NT T cells
pNB328-CD19CAR+pS328- α CD40	CD19CAR- α CD40 T cells

(continued)

Recombinant Plasmid	Name of Recombinant Cell
pNB328-CD19CAR-2A- α CD40	CD19CAR-2A- α CD40 T cells
pNB328- α CD40-IRES-CD 19CAR	α CD40-IRES-CD19CAR T cells
pNB328-CD19CAR	CD19CAR T cells
pNB328-CD19CAR+pS328- α CD40-wt	CD19CAR- α CD40-wt T cells
pNB328-mesoCAR+pS328- α CD40	mesoCAR- α CD40 T cells
pNB328-mesoCAR-2A- α CD40	mesoCAR-2A- α CD40 T cells
pNB328- α CD40-IRES-mesoCAR	aCD40-IRES-mesoCAR T cells
pNB328-mesoCAR	mesoCAR T cells
pNB328-mesoCAR+pS328- α CD40-wt	mesoCAR- α CD40-wt T cells
pNB328-EHCAR-EK-28TIZ+pS328- α CD40	EHCAR-EK-28TIZ- α CD40 T cells
pNB328-EHCAR-EK-28TIZ	EHCAR-EK-28TIZ T cells
pNB328-Muc1CAR+pS328- α CD40	Muc1CAR- α CD40 T cells
pNB328-Muc1CAR	Muc1CAR T cells
pS328-CD19CAR+pNB328-m279V	pS328-CD19CAR+pNB328-m279V T cells
pNB328-CD19CAR+pS328-m279V	CD19CAR-antiPD1 T cells
pNB328-CD19CAR-2A-m279V	pNB328-CD19CAR-2A-m279v T cells
pNB328-m279V-IRES-CD19CAR	pNB328-m279V-IRES-CD19CAR T cells
pNB328-CD19CAR+pS328-m279V-wt	CD19CAR-antiPD1-wt T cells
pNB328-mesoCAR+pS328-antiPD1	mesoCAR-antiPD1 T cells
pNB328-mesoCAR-2A-antiPD 1	mesoCAR-2A-antiPD1 T cells
pNB328-antiPD1-IRES-mesoCAR	antiPD1-IRES-mesoCAR T cells
pNB328-meso3CAR	meso3CAR T cells
pNB328-EHCAR-EK-28TIZ+pS328-antiPD1	EHCAR-EK-28TIZ-antiPD1 T cells
pS328-Muc1CAR+pNB328-m279V	pS328-Muc1CAR+pNB328-antiPD1 T cells
pNB328-Muc1CAR+pS328-m279V	Muc1CAR-antiPD1T cells
pNB328-Muc1CAR-2A-m279V	Muc1CAR-2A-antiPD1 T cells
pNB328-m279V-IRES-Muc1CAR	antiPD1-IRES-Muc1CAR T cells
pNB328-Muc1CAR+pS328-m279V-wt	pNB328-Muc1CAR+pS328-antiPD1-wt T cells
pNB328-EGFR-CAR+pS328- α CD47	α CD47-EGFR-CAR T cells
pNB328-EGFR-CAR	EGFR-CAR T cells
pNB328-EGFR-CAR+pS328-wt- α CD47	wt- α CD47-EGFR-CAR T cells
pNB328-meso3CAR+pS328- α CD47	α CD47-Meso3CAR T cells
pNB328-meso3CAR+pS328- α CD47-wt	wt- α CD47-Meso3CAR T cells

Example 3: Positive rate and antibody secretion of CAR-T cells

1. Detection of positive rate of CAR T cells by flow cytometry

[0147] CAR-T cells prepared in Example 2 were collected and divided into two groups each with 1×10^6 cells, washed twice with normal saline, and resuspended with 100ul normal saline. One group was added with 1ug of biotin-conjugated

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antigen (CD19, mesothelin, EGFR or mucin), the other was not. The two groups were incubated at 4°C for 30 minutes. The cells were washed twice with normal saline, resuspended with 100ul of normal saline again, added with 1ul of streptomycin-PE antibody, and incubated at 4°C for 30 minutes. The cells was washed twice with normal saline and assayed on the machine, with a control only being added with the secondary antibody. The results are shown in Table 3.

Table 3

T cell type	Positive Rate (%)
CD19CAR- α CD40 T cells	80.52
CD19CAR-2A- α CD40 T cells	1.21
α CD40-IRES-CD19CAR T cells	48.68
mesoCAR- α CD40 T cells	88.48
mesoCAR-2A- α CD40 T cells	23.02
aCD40-IRES-mesoCAR T cells	53.84
EH CAR-EK-28TIZ- α CD40 T cells	56.02
EH CAR-EK-28TIZ T cells	53.53
Muc 1CAR T cells	65.52
Muc1CAR- α CD40 T cells	71.48
CD19CAR T cells	50.24
CD19CAR-antiPD1 T cells	58.64
mesoCAR-antiPD1 T cells	86.42
mesoCAR-2A-antiPD1 T cells	68.01
antiPD1-IRES-mesoCAR T cells	46.97
EH CAR-EK-28TIZ-antiPD1 T cells	61.36
pS328-Muc1CAR+pNB328-antiPD1 T cells	45.63
Muc1CAR-antiPD1 T cells	91.17
Muc1CAR-2A-antiPD1 T cells	10.38
antiPD1-IRES-Muc1CAR T cells	35.33
α CD47-EGFR-CAR T cells	67.17
α CD47-Meso3CAR T cells	70.57

2. Detection of expression level of the antibody by CAR-T cells prepared in Example 2 using ELISA

[0148]

① Dilute the corresponding antigen (CD40, PD1 or CD47) to 0.5ug/ml with the coating solution (5ul + 1ml coating solution), coat the ELISA plate with 100ul/well overnight at 4°C.

② Wash 5 times with PBST, 3 minutes each time, dry the plate with absorbent paper by patting, 200ul/well.

③Add 100ul of blocking solution to each well, and incubate at 37°C for 1 hour.

④Wash 5 times with PBST, 3 minutes each time, dry the plate with absorbent paper by patting, 200ul/well.

⑤ Add samples and standards, 100ul/well, including replicates and control, and incubate at 37°C for 1 hour.

⑥ Wash 5 times with PBST, 3 minutes each time, dry the plate with absorbent paper by patting, 200ul/well.

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- ⑦ Dilute IgG F4 HRP with blocking solution at 1:30,000, 100ul/well, and incubate at 37°C for 45 minutes.
- ⑧ Wash 5 times with PBST, 3 minutes each time, dry the plate with absorbent paper by patting, 200ul/well.
- ⑨ Add chromogenic solution TMB, 100ul/well, incubate in dark at 37°C for 10-15min.
- ⑩ Add terminal solution to stop the reaction, 50ul/well.

[0149] The OD values at 450nm were measured by microplate reader, standard curves were generated, and concentrations of the CD40 antibody were calculated.

[0150] The results are shown in Table 4.

Table 4

Type of T cell	Type of Antibody	Secreted Amount (ng/ml)
CD19CAR- α CD40 T cell	mutant CD40 antibody	1437.1315
CD19CAR-2A- α CD40 T cell	mutant CD40 antibody	719.9956
α CD40-IRES-CD19CAR T cell	mutant CD40 antibody	543.3876
mesoCAR- α CD40 T cell	mutant CD40 antibody	1341.136
mesoCAR-2A- α CD40 T cell	mutant CD40 antibody	652.5344
aCD40-IRES-mesoCAR T cell	mutant CD40 antibody	525.2928
EHCAR-EK-28TIZ- α CD40 T cell	mutant CD40 antibody	238
EHCAR-EK-28TIZ T cell	mutant CD40 antibody	0.64
Muc1CAR T cell	mutant CD40 antibody	0.13
Muc1CAR- α CD40 T cell	mutant CD40 antibody	152
pS328-CD19CAR+pNB328-m279v T cell	mutant PD-1 antibody	840.9641
CD19CAR-antiPD1 T cell	mutant PD-1 antibody	1230.3335
pNB328-CD19CAR-2A-m279v T cell	mutant PD-1 antibody	100.00167
pNB328-m279V-IRES-CD19CAR T cell	mutant PD-1 antibody	410.75748
mesoCAR-antiPD1 T cell	mutant PD-1 antibody	1220.63764
mesoCAR-2A-antiPD1 T cell	mutant PD-1 antibody	675.58424
antiPD1-IRES-mesoCAR T cell	mutant PD-1 antibody	783.21583
EHCAR-EK-28TIZ-antiPD1 T cell	mutant PD-1 antibody	268
pS328-Muc1CAR+pNB328-antiPD1 T cell	mutant PD-1 antibody	45.93
Muc1CAR-antiPD1 T cell	mutant PD-1 antibody	91.17
Muc1CAR-2A-antiPD1 T cell	mutant PD-1 antibody	10.38
antiPD1-IRES-Muc1CAR T cell	mutant PD-1 antibody	35.33
α CD47-EGFR-CAR T cell	mutant CD47 antibody	1112.325
α CD47-Meso3CAR T cell	mutant CD47 antibody	986.549

Example 4: Tests of different plasmid ratios

[0151] CAR-T cells were prepared according to the method of Example 2, using the plasmid combinations constructed in Example 1 and mass ratios according to Table 5 (1ug+7ug, 2ug+6ug, 3ug+5ug, 4ug+4ug, 5ug+3ug, 6ug+2ug, 7ug+1ug). The method described in Example 3 was used to detect the positive rates and the antibody secretions of CAR T cells based on the different mass ratios (the method is the same as Example 3).

Table 5

pNB328-CD19CAR	pS328- α CD40
pNB328-mesoCAR	pS328- α CD40
pNB328-EHCAR-EK-28TIZ	pS328- α CD40
pNB328-Muc1CAR	pS328- α CD40
pNB328-CD19CAR	pS328-m279V
pNB328-mesoCAR	pS328-antiPD 1
pNB328-EHCAR-EK-28TIZ	pS328-antiPD1
pNB328-Muc1CAR	pS328-m279V
pNB328-EGFR-CAR	pS328- α CD47
pNB328-meso3CAR	pS328- α CD47

[0152] Exemplary results are shown in Tables 6 and 7 below.

Table 6

Different mass ratios (pNB328-CD19CAR: pS328-m279V)	Expression of PD1 antibody (ng/ml)	Positive rate of CAR-T cells (%)
4:4	1320.93	80.89
3:5	1130.73	72.07
2:6	1030.02	57.72
1:7	420.93	13.01

Table 7

Different mass ratios (pNB328-Muc1CAR: pS328-m279V)	Expression of PD1 antibody (ng/ml)	Positive rate of CAR-T cells (%)
1:1	2200.01	92.55
3:5	1860.00	79.54
1:3	1260.77	54.63
1:7	310.88	20.82

[0153] The results show that, based on the positive rate and antibody secretion results, EHCAR-EK-28TIZ- α CD40 T cells prepared by 5ug pNB328-EHCAR-EK-28TIZ and 3ug pS328- α CD40 have the best effect (positive rate greater than 60%, antibody secretion greater than 230ng/ml); in other plasmid combinations, 4ug+4ug has better effect than other mass ratios.

Example 5: Comparison of cytokine release between CD19CAR and CD19CAR- α CD40 T cells under specific stimulation of CD19 antigen

[0154] 96-well plates were coated overnight with 2ug/ml CD19 antigen at 4°C, washed 3 times with PBS, added with 1×10^5 CD19CAR and CD19CAR- α CD40 T cells prepared according to Example 2 and control Mock T cells. Supernatants were collected after 24 hours of culture. BD's CBA Human Th1/Th2 Cytokine Kit II was used to detect the cytokine secretion of these three T cells upon stimulation by CD19 antigen. The particular steps are as follows:

(1) Mix human IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ capture magnetic beads by vortex, add 50ul of mixed beads to each tube;

- (2) Add 50ul of human Th1/Th2 cytokine standard (diluted to 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156pg/ml, 80pg/ml, 40pg/ml, 20pg/ml, or Opg/ml) and 50ul of the sample to be tested (diluted by 2-fold with the diluents);
- (3) Add 50ul of human Th1/Th2-II-PE detection antibody to each tube;
- (4) Incubate at room temperature in the dark for 3h;
- (5) Add 1ml of washing buffer to each tube, centrifuge at 200 for 5min, and discard the supernatant;
- (6) Add 300ul of washing buffer to each tube to resuspend the cells, and transfer the cells to a flow cytometry tube to detect the fluorescence value by a flow cytometer.

[0155] The results are shown in Figure 1.

Example 6: Proliferation detection of CD19CAR and CD19CAR- α CD40 T cell

[0156] 3×10^5 cells of Mock T cells and the CD19CAR T cells and CD19CAR- α CD40 T cells that have been cultured for 8 days according to Example 2 were cultured in 12-well plates with the culture volume of 1 ml.

2. 100 μ L of cell-containing culture medium from each group was added to different wells of a 96-well white opaque plate, with culture medium without cells as a blank control. Each well was added with 100 μ L CellTiter-Glo reagent, mixed on a shaker for 2 min, and incubated at room temperature for 10 min, and then detected by a microplate reader for fluorescence value of Luc. The used CellTiter-Glo Luminescent Cell Viability Assay kit was purchased from Promega.

3. The same detections for the cells from 12-well plates were made according to the above steps on the 9th, 10th, 11th, 12th, and 13th days of culture. Cell proliferation curves were drawn based on the detected fluorescence values.

[0157] The results show that CD19CAR- α CD40 T cells have a better proliferation than CD19CAR T cells. The results are shown in Figure 2.

Example 7: Functional assay of CD19CAR T cells and CD19CAR- α CD40 T cells *in vivo*

[0158] Twelve of 4-6 weeks old NSG completely immunodeficient mice, with an average weight of 22-27g, were provided by Beijing Biocytogen Biotech Co., Ltd., and raised by a SPF animal laboratory.

[0159] Human B-cell lymphoma Raji-luc cells in logarithmic growth phase cultured *in vitro* were centrifuged, collected and resuspended in PBS solution. The cells were centrifuged at 3000g for 2 minutes at room temperature, the supernatant was discarded and the cells were resuspended in PBS solution, centrifuged and collected, and the concentration of the cell suspension was adjusted to 5×10^7 cells/ml. The Raji-luc cells were inoculated subcutaneously in the dorsum of the right rib of the mouse at 0.1 ml/mouse. About 10 days after the inoculation, the size of the tumor was observed by an *in vivo* imager, and the NSG immunodeficient mice were randomly divided into 5 groups: PBS group, Mock T group, CD19CAR T group, CD19CAR- α CD40-wt T, and CD19CAR- α CD40T group. Each group was injected through the tail vein with corresponding T cells (from Example 2) at 1×10^7 cells/100ul, and PBS group was injected with 100ul of PBS. The living conditions of mice were observed every day and the change of the tumor in each mouse was observed by *in vivo* imager every 7-8 days.

[0160] The results are shown in Figure 3.

Example 8: Comparison of the killing effect by mesoCAR T and mesoCAR- α CD40 T cells

[0161] Real-time label-free cell analysis system was used to detect the killing effect of mesoCAR T cells and mesoCAR- α CD40 T cells prepared in Example 2 on tumor cells *in vitro*.

[0162] Specifically, target cells and effector cells that match MHC class I were selected, and Real-time label-free cell analysis system(RTCA, ACEA) was used to detect the killing effect of the above two CAR-T cells *in vitro*. The steps are as follows:

- (1) Zero adjustment: Add 50 μ l DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;
- (2) Target cell plating: Plate cervical cancer cell Hela, ovarian cancer cell SK-OV-3, gastric cancer cell HGC-27 (American Type Culture Collection ATCC) at 10^4 cells/50 μ l per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;
- (3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50 μ l per well, with the effect target ratio of 4:1 and Mock T cells as the control, start step 3 to continue co-cultivation for 24h, then

generate the cell proliferation curve;

[0163] The results are shown in Figure 4. The killing effect of the mesoCAR- α CD40 T cells that self-express CD40 antibodies are substantially the same with that of the mesoCAR T cells alone. The expression of antibody does not affect the CAR-T function.

Example 9: Comparison of cytokine release between mesoCAR and mesoCAR- α CD40 T cells under specific stimulation of mesothelin antigen

[0164] 96-well plates were coated overnight with 2 μ g/ml mesothelin antigen at 4°C, washed 3 times with PBS, added with 1 $\times$ 10⁵ meso3CAR T cells and mesoCAR- α CD40 T cells prepared according to Example 2 and control Mock T cells. Supernatants were collected after 24 hours of culture. BD's CBA Human Th1/Th2 Cytokine Kit II was used to detect the cytokine secretion of these three T cells upon stimulation by mesothelin antigen. The particular steps are as follows:

- (1) Mix human IL-2, IL-4, IL-6, IL-10, TNF, IFN- γ capture magnetic beads by vortex, add 50ul of mixed beads to each tube;
- (2) Add 50ul of human Th1/Th2 cytokine standard (diluted to 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156pg/ml, 80pg/ml, 40pg/ml, 20pg/ml, or Opg/ml) and 50ul of the sample to be tested (diluted by 2-fold with the diluents);
- (3) Add 50ul of human Th1/Th2-II-PE detection antibody to each tube;
- (4) Incubate at room temperature in the dark for 3h;
- (5) Add 1ml of washing buffer to each tube, centrifuge at 200 for 5min, and discard the supernatant;
- (6) Add 300ul of washing buffer to each tube to resuspend the cells, and transfer the cells to a flow cytometry tube to detect the fluorescence value by a flow cytometer.

[0165] The results are shown in Figure 5. There is no significant difference in the amount of cytokine secretion between mesoCAR- α CD40 T cells that self-express CD40 antibody and mesoCAR T cells alone.

Example 10: Proliferation detection of mesoCAR and mesoCAR- α CD40 T cell

[0166] 3 $\times$ 10⁵ cells of Mock-T cells and the mesoCAR T cells and mesoCAR - α CD40 T cells that have been cultured for 8 days according to Example 2 were cultured in 12-well plates in a culture volume of 1 ml.

2. 100 μ L of cell-containing culture medium from each group was added to different wells of a 96-well white opaque plate, with culture medium without cells as a blank control. Each well was added with 100 μ L CellTiter-Glo reagent, mixed on a shaker for 2 min, and incubated at room temperature for 10 min, and then detected by a microplate reader for fluorescence value of Luc. The used CellTiter-Glo Luminescent Cell Viability Assay kit was purchased from Promega.

3. The same detections for the cells from 12-well plates were made according to the above steps on the 9th, 10th, 11th, 12th, and 13th days of culture. Cell proliferation curves were drawn based on the detected fluorescence values.

[0167] The results are shown in Figure 6. MesoCAR- α CD40 T cells have better proliferation than mesoCAR T cells.

Example 11: The therapeutic effect of mesoCAR and mesoCAR- α CD40 T cells on ovarian cancer mouse xenograft model

[0168]

1: Twenty of 4-6 weeks old NSG completely immunodeficient mice, with an average weight of 22-27g, were provided by Beijing Biocytogen Biotech Co., Ltd., and raised by a SPF animal laboratory.

2: Adhered human ovarian cancer cells SK-OV-3-luc in logarithmic growth phase cultured *in vitro* were digested with 0.25% trypsin, centrifuged, collected and resuspended in PBS solution. The cells were centrifuged at 1000rpm for 2 minutes at room temperature, the supernatant was discarded and the cells were resuspended in PBS solution, centrifuged and collected, and the concentration of the cell suspension was adjusted to 5 $\times$ 10⁷ cells/ml.

3: The OVCAR-3-luc cells were inoculated subcutaneously in the dorsum of the right rib of the mouse at 0.1 ml/mouse. 7 days after the inoculation, the size of the tumor was observed by an *in vivo* imager, the tumor size was measured by a vernier caliper, and the NSG immunodeficient mice were randomly divided into 5 groups: PBS group, and Mock T group, mesoCAR T group, mesoCAR- α CD40-wt T, and mesoCAR- α CD40 T group prepared according to Example

2. Each group was injected through the tail vein with corresponding T cells at 1×10^7 cells/100ul, and PBS group was injected with 100ul of PBS.

4: The living conditions of mice were observed every day and the change of the tumor in each mouse was observed by in vivo imager every 4 days.

[0169] The results are shown in Figure 7.

Example 12: Comparison of the proliferation rate of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells

[0170] 3×10^5 cells of Mock-T cells and the EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ- α CD40 T cells that have been cultured for 8 days according to Example 2 were cultured in 12-well plates in a culture volume of 1 ml. 80 μ L of cell-containing culture medium from each group was added to different wells of a 96-well white opaque plate, with 80 μ L nutrient solution further added to the original 12-well plate. The 96-well plate was added with 80 μ L CellTiter-Glo reagent, mixed on a shaker for 2 min, and incubated at room temperature for 10 min, and then detected by a microplate reader for fluorescence value of Luc. The used CellTiter-Glo Luminescent Cell Viability Assay kit was purchased from Promega. The same detections for the cells from 12-well plates were made according to the above steps on the 9th, 10th, 11th, 12th, and 13th days of culture. Cell proliferation curves were drawn based on the detected fluorescence values.

[0171] The results are shown in Figure 8. The proliferation rate of EHCAR-EK-28TIZ- α CD40 T cells is significantly higher than that of EHCAR-EK-28TIZ T cells, indicating that the expression of CD40 antibody can promote the proliferation of CAR-T cells.

Example 13: Cell phenotype analysis of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells

[0172] The EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells obtained in Example 2 were added into six 1.5ml EP tubes with 1×10^6 cells/tube, washed twice with PBS, and centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. Two of the tubes were added with the flow cytometry antibodies anti-CD 107 α -PE and anti-CD69-PE to detect activated T cell phenotype; one of the tubes was added with the flow cytometry antibodies anti-CD45RO-PECy5+ anti-CD 197-FITC+ anti-CD62L-PE to detect memory T cell phenotype; one of the tubes was added with the flow cytometry antibody anti-PD1-PE to detect the inhibitory T cell phenotype; and the other 2 tubes were added with the isotype control flow cytometry antibodies IgG1-PE and IgG1-PE+IgG2a-PECy5+IgG2a-PE; 2 μ L for each antibody (Jackson ImmunoResearch). The precipitate was flicked to make it mix evenly; the cells were incubated at room temperature in the dark for 30 min, washed with PBS once, and centrifuged at 1200 rpm for 5 min, the supernatant was discarded and 400 μ L of normal saline was added, and the cells were transferred to a flow tube, and analyzed on the machine.

[0173] The results show that the expressions of the aging phenotype PD1 of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells by flow cytometry are similar (Figure 9A); the expressions of the activated phenotypes CD69 and CD107 α of EHCAR-EK-28TIZ- α CD40 T cells are higher than those of EHCAR-EK-28TIZ cells (Figures 9B and 9C); meanwhile, CD62L (L-selectin) is a marker of central memory T cells, and CD197 is a marker of effector memory T cells, and the proportion of effector T cells in EHCAR-EK-28TIZ- α CD40 T cells is significantly higher than those of EHCAR-EK-28TIZ cells and Mock-T cells (Figure 9D). These results indicate that the expression of CD40 antibody can promote the activation of CAR-T cells and enhance their immune killing effect.

Example 14: Comparison of the killing effect of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells

[0174] Target cells and effector cells that match MHC class I were selected, and Real-time label-free cell analysis system(RTCA, ACEA) was used to detect the *in vitro* killing effect of the EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ- α CD40 T cells obtained in Example 2. The steps are as follows:

(1) Zero adjustment: Add 50 μ L DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;

(2) Target cell plating: Plate human liver cancer cell HCCLM3, human lung degenerative cancer cell Calu-6 and human non-small cell lung cancer H23 (American Type Culture Collection ATCC) at 10^4 cells/50 μ L per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;

(3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50 μ L per well, with the effect target ratio of 4:1 and Mock T cells transferred with empty pNB328 as the control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve;

[0175] The results are shown in Figure 10. The killing effect of EHCAR-EK-28TIZ- α CD40 T cells that self-express

CD40 antibody on a variety of tumor cells is significantly greater than that of EHCAR-EK-28TIZ T cells and control T cells.

Example 15: Comparison of cytokine release between EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ- α CD40 T cells under specific stimulation of EGFR antigen

[0176] 96-well plates were coated overnight with 5 μ g/ml EGFR antigen at 4°C, washed 3 times with PBS, added with 1×10^5 (100 μ l of volume) EHCAR-EK-28TIZ and EHCAR-EK-28TIZ- α CD40 T cells prepared according to Example 2 and control Mock T cells (transferred with empty pNB328). Supernatants were collected after 24 hours of culture. The cytokine secretion of these three T cells after being stimulated by EGFR antigen was detected.

[0177] The results are shown in Figure 11. The secretions of IL-2 and IFN- γ in EHCAR-EK-28TIZ- α CD40 are significantly higher than those of EHCAR-EK-28TIZ T cells and Mock-T, indicating that self-expressing CD40 agonistic antibodies can promote CAR-T cells to secrete cytokines.

Example 16: Anti-tumor effect of EHCAR-EK-28TIZ T cells, EHCAR-EK-28TIZ- α CD40-wt T cells and EHCAR-EK-28TIZ- α CD40 T cells *in vivo*

[0178] Twenty NSG mice of 4-6 weeks were divided into 5 groups on average, with 4 mice of each group inoculated with liver cancer cell line HCCLM3-LUC for 1×10^7 per mouse. 10 days after tumor being formed, each group was injected via tail vein with PBS (100 μ l PBS), Mock-T cells, EHCAR-EK-28TIZ T cells, EHCAR-EK-28TIZ- α CD40 T cells and EHCAR-EK-28TIZ- α CD40-wt T cells obtained in Example 2 (1×10^7 /100 μ l of each), respectively. The changes of the tumor fluorescence in mice were observed and recorded.

[0179] The results show that PBS, Mock-T, EHCAR-EK-28TIZ- α CD40-wt T cells have no therapeutic effect on the tumor model, EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ- α CD40 T cells have anti-tumor effects, and EHCAR-EK-28TIZ- α CD40 T cells are significantly better. The details are shown in Figure 12.

Example 17: Comparison of the proliferation rate of Muc1CAR T and Muc1CAR- α CD40 T cells

[0180] 3×10^5 cells of Mock-T cells and the Muc1CAR T cells and Muc1CAR- α CD40 T cells that have been cultured for 8 days according to Example 2 were cultured in 12-well plates in a culture volume of 1 ml. 80 μ L of cell-containing culture medium from each group was added to different wells of a 96-well white opaque plate, with 80 μ L nutrient solution further added to the original 12-well plate. The 96-well plate was added with 80 μ L CellTiter-Glo reagent, mixed on a shaker for 2 min, and incubated at room temperature for 10 min, and then detected by a microplate reader for fluorescence value of Luc. The used CellTiter-Glo Luminescent Cell Viability Assay kit was purchased from Promega. The same detections for the cells from 12-well plates were made according to the above steps on the 9th, 10th, 11th, 12th, and 13th days of culture. Cell proliferation curves were drawn based on the detected fluorescence values.

[0181] The results are shown in Figure 13. The proliferation rate of Muc1CAR- α CD40 T cells is significantly higher than that of Muc1CAR T cells, indicating that the expression of CD40 antibody can promote the proliferation of CAR-T cells.

Example 18: Cell phenotype analysis of Muc1CAR T and Muc1CAR- α CD40 T cells

[0182] The Muc1CAR and Muc1CAR- α CD40 T cells obtained in Example 2 were added into seven 1.5ml EP tubes with 1×10^6 cells/tube, washed twice with PBS, and centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. One of the tubes was added with the flow cytometry antibody anti-CD25-PE to detect activated T cell phenotype; one of the tubes was added with the flow cytometry antibodies anti-CD45RO-PECy5+ anti-CD 197-FITC+ anti-CD62L-PE to detect memory T cell phenotype; two of the tubes were added with the flow cytometry antibodies anti-PD1-PE and anti-LAG3-Alexa Fluor 647 to detect the inhibitory T cell phenotype; and the other 3 tubes were added with the isotype control flow cytometry antibodies IgG1-PE, IgG1-PE+IgG2a-PECy5+IgG2a-PE and IgG1 Alexa Fluor 647; 2 μ l for each antibody (Jackson ImmunoResearch). The precipitate was flicked to make it mix evenly, the cells were incubated at room temperature in the dark for 30 min, washed with PBS once, and centrifuged at 1200 rpm for 5 min, the supernatant was discarded and 400 μ l of normal saline was added, and the cells were transferred to a flow tube, and analyzed on the machine.

[0183] The results showed that the expressions of the aging phenotypes PD1 and LAG3 of Muc1CAR- α CD40 T cells by flow cytometry are lower than that of Muc1CAR T cells, and the expression of the activated phenotype CD25 of Muc1CAR- α CD40 T cells is higher than that of Muc1CAR T cells (Figures 14A); meanwhile, CD62L (L-selectin) is a marker of central memory T cells, and CD197 is a marker of effector memory T cells, and the proportion of effector T cells in Muc1CAR- α CD40 T cells is significantly higher than those of Muc1CAR T cells and Mock-T cells (Figure 14B). These results indicate that the expression of CD40 antibody can promote the activation of CAR-T cells and enhance their immune killing effect.

Example 19: Comparison of the killing effect by Muc1CAR T and Muc1CAR- α CD40 T cells

[0184] Target cells and effector cells that match MHC class I were selected, and Real-time label-free cell analysis system (RTCA, ACEA) was used to detect the *in vitro* killing effect of the Muc1CAR T cells and Muc1CAR- α CD40 T cells obtained in Example 2. The steps are as follows:

- (1) Zero adjustment: add 50 μ l DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;
- (2) Target cell plating: Plate human liver cancer cell HCCLM3 and human non-small cell lung cancer H23 (American Type Culture Collection ATCC) at 10⁴ cells/50 μ l per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;
- (3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50 μ l per well, with the effect target ratio of 4:1 and Mock T cells transferred with empty pNB328 as the control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve.

[0185] The results are shown in Figure 15. The killing effect of Muc1CAR- α CD40 T cells that self-express CD40 antibody on a variety of tumor cells is significantly greater than those of Muc1CAR T cells and control T cells.

Example 20: Comparison of cytokine release between Muc1CAR T and Muc1CAR- α CD40 T cells under specific stimulation of Muc1 antigen

[0186] 96-well plates were coated overnight with 5ug/ml Muc1 antigen at 4°C, washed 3 times with PBS, added with 1 $\times$ 10⁵ (100ul of volume) Muc1CAR T cells and Muc1CAR- α CD40 T cells prepared according to Example 2 and control Mock T cells (transferred with empty pNB328). Supernatants were collected after 24 hours of culture. The cytokine secretion of these three T cells after being stimulated by Muc1 antigen was detected.

[0187] The results are shown in Figure 16. The secretions of IL-2, TNF α and IFN- γ by Muc1CAR- α CD40 T cells are significantly higher than those by Muc1CAR T cells and Mock-T, indicating that self-expressing CD40 agonistic antibodies can promote the secretion of cytokines by CAR-T cells.

Example 21: Anti-tumor effect of Muc1CAR-T, Muc1CAR- α CD40-wt T cells and Muc1CAR- α CD40 T cells *in vivo*

[0188] Twenty NSG mice of 4-6 weeks were divided into 5 groups on average, with 4 mice of each group inoculated with liver cancer cell line HCCLM3-LUC for 1 $\times$ 10⁷ per mouse. 10 days after tumor being formed, each group was injected via tail vein with PBS (100ul PBS), Mock T cells, Muc1CAR-T cells, Muc1CAR- α CD40 T cells and Muc1CAR- α CD40-wt T cells obtained in Example 2 (1 $\times$ 10⁷/100ul of each), respectively. The changes of the tumor fluorescence in mice were observed and recorded.

[0189] The results show that PBS, Mock-T, Muc1CAR- α CD40-wt T cells have no therapeutic effect on the tumor models, and Muc1CAR-T and Muc1CAR- α CD40 T cells have anti-tumor effects, with Muc1CAR- α CD40 T cells having significantly better effects. The details are shown in Figure 17.

Example 22: Killing assay of Mock T cells, CD19CAR T cells, and CD19CAR-antiPD1 T cells on the cultured tumor cells *in vitro*

[0190] Target cells and effector cells that match MHC class I were selected, and DELFIA EuTDA Cytotoxicity Test was used to detect the killing effect of the CAR T cells *in vitro*. The steps are as follows:

- (1) Centrifuge, collect Raji cells, and wash once with PBS;
- (2) Centrifuge, collect cell pellet, resuspend the cells in 1640 medium and count cell number, adjust the cell density to 1 $\times$ 10⁶/ml;
- (3) Add 5ul of fluorescence-enhancing ligand in 2-4ml of the above cells, then place the cells in 37°C, 5% CO₂ cell incubator for 20min;
- (4) Wash the cells 3-5 times with PBS;
- (5) Centrifuge, collect the cell pellet and resuspend the cells in 1640 medium and count cell number, adjust the cell density to 5 $\times$ 10⁴/ml, and add 100ul of the cell suspension to a 96-well culture plate.
- (6) Count cell numbers for the Mock T cells, CD19CAR T cells and CD19CAR-antiPD1 T cells prepared in Example 2, add 100ul of the cell suspensions to the above Raji cells according to different effective target ratios 4:1, with a high control group (tumor cells lysed by lysis buffer), a low control group (only tumor cells), a blank control group (only medium);

- (7) Place in 37°C, 5% CO₂ cell incubator for 20min and co-culture for 3h;
- (8) Transfer 20ul culture supernatant to 96-well whiteboard;
- (9) Add 200ul Europium solution;
- (10) Shake at room temperature for 15 minutes;
- (11) Generate readings by time-resolved fluorescence detection in microplate reader;

[0191] The results are shown in Figure 18. CD19CAR T cells and CD19CAR-antiPD1 T have strong and comparable killing effects on tumor cells.

Example 23: Detection of differences of activation phenotype and cytokine secretion between Mock T cells, CD19CAR T cells and CD19CAR-antiPD1 T cell by flow cytometry

[0192]

1. The suspended Mock T cells, CD19CAR T cells and CD19CAR-antiPD1 T cells prepared in Example 2 were washed twice with PBS, centrifuged at 1200rpm for 5min, and added with 2ul of the isotype control antibody IgG1-PE, the fluorescent flow cytometry antibodies anti-CD69-PE, anti-KLRG1-PE, anti-PD1-PE; isotype control antibody IgG1-PC5, the fluorescent flow cytometry antibody anti-CD107-PC5; isotype control antibody IgG1 FITC, fluorescent flow cytometry antibody anti-CD62L-FITC; isotype control antibody IgG1-PC5, fluorescent flow cytometry antibody anti-CD45RO-PC5; isotype control antibody IgG1-PE, fluorescent flow cytometry antibody anti-CCR7-PE. The precipitate was flicked to make it mix evenly, the cells were incubated at room temperature in the dark for 30 min, and washed with PBS once, 400 μ l PBS was added and the cells were transferred to a flow tube, and then assayed on the machine.

The results are shown in Figure 19 (panels 1 and 2). The PD-1 single-chain antibody secreted by CD19CAR-antiPD1 T cells can block the PD-1 protein on the surface of T cells well. CD19CAR T cells and CD19CAR-antiPD1 T cells have obvious killing activity *in vitro* and can also promote the formation of memory T, while activation marker CD69 is significantly higher than Mock T cells and depletion marker LAG3 is significantly lower than Mock T cells.

2. 24-well plates were coated overnight with 5ug/ml CD19 antigen at 4°C, washed 3 times with PBS, added with 3×10^5 Mock T cells, CD19CAR T cells and CD19CAR-antiPD1 T cells. Supernatants were collected after 24 hours of culture. BD™CBA Human Th1/Th2 Cytokine Kit II was used to detect the secretion of cytokines of CD19CAR T cells and CD19CAR-antiPD1 T cells after stimulated by CD19 antigen:

(1) Mix human IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ capture magnetic beads by vortex, add 50ul of mixed beads to each tube;

(2) Add 50ul of human Th1/Th2 cytokine standard (diluted to 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156pg/ml, 80pg/ml, 40pg/ml, 20pg/ml, or Opg/ml) and 50ul of the sample to be tested (diluted by 2-fold with the diluents);

(3) Add 50ul of human Th1/Th2-II-PE detection antibody to each tube;

(4) Incubate at room temperature in the dark for 3h;

(5) Add 1ml of washing buffer to each tube, centrifuge at 200 for 5min, and discard the supernatant;

(6) Add 300ul of washing buffer to each tube to resuspend the cells, and transfer the cells to a flow cytometry tube to detect the fluorescence value by a flow cytometer.

[0193] The results are shown in Figure 19 (the third panel). The secretions of IL-2, TNF- α and IFN- γ of CD19CAR T cells and CD19CAR-antiPD1 T cells are greatly improved as compared to Mock T cells. The secretions of IL-4, IL-6 and IL-10 of the three cells are not substantially different.

[0194] 3. 1×10^6 CD19CAR T cells and CD19CAR-antiPD1 T cells were added to 1.5ml EP tubes respectively, washed twice with PBS, centrifuged at 1200rpm for 5min, added with 2ul of α -CD3CD4CD8 antibody, incubated at room temperature in the dark for 30min, and washed once with PBS, 400ul PBS was added, and the cells were transferred to the flow tube, and assayed on the machine.

[0195] The results are shown in Figure 19 (the fourth panel). No big difference is shown in the percentages of CD3⁺CD4⁺ and CD3⁺CD8⁺ cells in CD19CAR T cells, CD19CAR-antiPD1 T cells and Mock T cells.

Example 24: Functional assay of CD19CAR T cells, CD19CAR-antiPD1 T cells and CD19CAR-antiPD1-wt T cells *in vivo*

[0196] This example used twelve of 4-6 weeks old NSG completely immunodeficient mice, with an average weight of 22-27g, provided by Beijing Biocytogen Biotech Co., Ltd., and raised by a SPF animal laboratory.

[0197] Human B-cell lymphoma Raji-luc cells in logarithmic growth phase cultured *in vitro* were centrifuged, collected

and resuspended in PBS solution. The cells were centrifuged at 3000g for 2 minutes at room temperature, the supernatant was discarded and the cells were resuspended in PBS solution, centrifuged and collected, and the concentration of the cell suspension was adjusted to 5×10^7 cells/ml. The Raji-luc cells were inoculated subcutaneously in the dorsum of the right rib of the mouse at 0.1 ml/mouse. About 10 days after the inoculation, the size of the tumor was observed by an *in vivo* imager, and the NSG immunodeficient mice were randomly divided into 4 groups: PBS group, Mock T group, CD19CAR T group, CD19CAR-antiPD1 T, and CD19CAR-antiPD1-wt T group (T cells prepared according to Example 2). Each group was injected through the tail vein with corresponding T cells at 1×10^7 cells/100ul, and PBS group was injected with 100ul of PBS. The living conditions of mice were observed every day and the change of the tumor in each mouse was observed by *in vivo* imager every 7-8 days.

[0198] The results are shown in Figure 20.

Example 25: Comparison of the killing effect by meso3CAR T and mesoCAR-antiPD1 T cells

[0199] Real-time label-free cell analysis system was used to detect the killing effect of meso3CAR T cells and mesoCAR-antiPD1 T cells prepared in Example 2 on tumor cells *in vitro*.

[0200] Specifically, target cells and effector cells that match MHC class I were selected, and Real-time label-free cell analysis system (RTCA, ACEA) was used to detect the killing effect of the above two CAR-T cells *in vitro*. The steps are as follows:

- (1) Zero adjustment: Add 50μl DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;
- (2) Target cell plating: Plate cervical cancer cell Hela, ovarian cancer cell SK-OV-3, gastric cancer cell HGC-27 (American Type Culture Collection ATCC) at 10^4 cells/50μl per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;
- (3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50μl per well, with the effect target ratio of 4:1 and Mock T cells without a transferred plasmid as a control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve.

[0201] The results are shown in Figure 21. The mesoCAR-antiPD1 T cells that self-express PD1 antibodies have substantially the same killing effect with meso3CAR T cells alone. The expression of antibody does not affect the CAR-T function.

[0202] Example 26: Comparison of cytokine release between meso3CAR and mesoCAR-antiPD1 T cells under specific stimulation of mesothelin antigen

[0203] 96-well plates were coated overnight with 2ug/ml mesothelin antigen at 4°C, washed 3 times with PBS, added with 1×10^5 meso3CAR and mesoCAR-antiPD1 T cells prepared according to Example 2 and control Mock T cells. Supernatant was collected after 24 hours of culture. BD™CBA Human Th1/Th2 Cytokine Kit II was used to detect the cytokine secretion of these three T cells upon stimulation by mesothelin antigen. The particular steps are as follows:

- (1) Mix human IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ capture magnetic beads by vortex, add 50ul of mixed beads to each tube;
- (2) Add 50ul of human Th1/Th2 cytokine standard (diluted to 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156pg/ml, 80pg/ml, 40pg/ml, 20pg/ml, or Opg/ml) and 50ul of the sample to be tested (diluted by 2-fold with the diluents);
- (3) Add 50ul of human Th1/Th2-II-PE detection antibody to each tube;
- (4) Incubate at room temperature in the dark for 3h;
- (5) Add 1ml of washing buffer to each tube, centrifuge at 200 for 5min, and discard the supernatant;
- (6) Add 300ul of washing buffer to each tube to resuspend the cells, and transfer the cells to a flow cytometry tube to detect the fluorescence value by a flow cytometer.

[0204] The results are shown in Figure 22. There is no significant difference in the amount of cytokine secretion between mesoCAR-antiPD1 T cells that self-express PD1 antibody and meso3CAR T cells alone.

Example 27: The therapeutic effect of meso3CAR and mesoCAR-antiPD1 T cells on ovarian cancer mouse xenograft model

[0205]

1. 25 of 4-6 weeks old NSG completely immunodeficient mice, with an average weight of 22-27g, were provided by

Beijing Biocytogen Biotech Co., Ltd., and raised by a SPF animal laboratory.

2. Adhered human ovarian cancer cells SK-OV-3-luc in logarithmic growth phase cultured *in vitro* were digested with 0.25% trypsin, centrifuged, collected and resuspended in PBS solution. The cells were centrifuged at 1000rpm for 2 minutes at room temperature, the supernatant was discarded and the cells were resuspended in PBS solution,

centrifuged and collected, and the concentration of the cell suspension was adjusted to 5×10^7 cells/ml. 3. The SK-OV-3-luc cells were inoculated subcutaneously in the dorsum of the right rib of the mouse at 0.1 ml/mouse. 7 days after the inoculation, the fluorescence intensity was observed by an *in vivo* imager, and the NSG immunodeficient mice were randomly divided into 5 groups. Each group was injected through the tail vein with corresponding T cells at 1×10^7 cells/100ul, and PBS group was administrated with 100ul of PBS.

4. The living conditions of mice were observed every day and the change of the tumor in each mouse was observed by *in vivo* imager every 4 days.

[0206] The results are shown in Figure 23. In the SK-OV-3 ovarian cancer mouse xenograft model, MesoCAR-antiPD1 T cells have a significantly better therapeutic effect than Meso3CAR T cells, while Meso3CAR-wt-antiPD1 T cells have substantially no effect.

Figure 28: Comparison of the proliferation rate of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells

[0207] 3×10^5 cells of Mock-T cells and the EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ-antiPD1 T cells that have been cultured for 8 days according to Example 2 were cultured in 12-well plates in a culture volume of 1 ml. 80 μ L of cell-containing culture medium from each group was added to different wells of a 96-well white opaque plate, with 80 μ L nutrient solution further added to the original 12-well plate. The 96-well plate was added with 80 μ L CellTiter-Glo reagent, mixed on a shaker for 2 min, and incubated at room temperature for 10 min, and then detected by a microplate reader for fluorescence value of Luc. The used CellTiter-Glo Luminescent Cell Viability Assay kit was purchased from Promega. The same detections for the cells from 12-well plates were made according to the above steps on the 9th, 10th, 11th, 12th, and 13th days of culture. Cell proliferation curves were drawn based on the detected fluorescence values.

[0208] The results are shown in Figure 24. The proliferation rate of EHCAR-EK-28TIZ-antiPD1 T cells is significantly higher than that of EHCAR-EK-28TIZ T cells, indicating that the expression of CD40 antibody can promote the proliferation of CAR-T cells.

Figure 29: Cell phenotype analysis of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells

[0209] The EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ-antiPD1 T cells obtained in Example 2 were added into six 1.5ml EP tubes with 1×10^6 cells/tube respectively, washed twice with PBS, centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. Two of the tubes were added with the flow cytometry antibodies anti-CD107 α -PE and anti-CD69-PE to detect activated T cell phenotype; one of the tubes was added with the flow cytometry antibodies anti-CD45RO-PECy5+ anti-CD 197-FITC+ anti-CD62L-PE to detect memory T cell phenotype, one of the tubes was added with the flow cytometry antibody anti-PD1-PE to detect the inhibitory T cell phenotype, and the other 2 tubes were added with the isotype control flow cytometry antibodies IgG1-PE and IgG1-PE+IgG2a-PECy5+IgG2a-PE; 2 μ L for each antibody (Jackson ImmunoResearch). The precipitate was flicked to make it mix evenly. After incubation at room temperature in the dark for 30 min, the cells were washed with PBS once, centrifuged at 1200 rpm for 5 min, the supernatant was discarded and 400 μ L of normal saline was added, and the cells were transferred to a flow tube, and analyzed on the machine.

[0210] The results show that the expression of the aging phenotype PD1 of EHCAR-EK-28TIZ-antiPD1 T cells by flow cytometry is significantly lower than that of EHCAR-EK-28TIZ (Figure 25A); the expressions of the activated phenotypes CD69 and CD107 α of EHCAR-EK-28TIZ-antiPD1 T cells are higher than those of EHCAR-EK-28TIZ cells (Figures 25B and 25C); meanwhile, CD62L (L-selectin) is a marker of central memory T cells, and CD197 is a marker of effector memory T cells, and the proportion of effector T cells in EHCAR-EK-28TIZ-antiPD1 T cells is significantly higher than those of EHCAR-EK-28TIZ cells and Mock-T cells (Figure 25D). These results indicate that the expression of PD1 antibody can inhibit the depletion of CAR-T cells, promote the activation of CAR-T cells, and enhance their immune killing effect.

Figure 30: Comparison of the killing effect of EHCAR-EK-28TIZ and EHCAR-EK-28TIZ-antiPD1 T cells

[0211] Target cells and effector cells that match MHC class I were selected, and Real-time label-free cell analysis system (RTCA, ACEA) was used to detect the *in vitro* killing effect of the EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ-antiPD1 T cells obtained in Example 2. The steps are as follows:

(1) Zero adjustment: Add 50 μ l DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;

(2) Target cell plating: Plate human liver cancer cell HCCLM3, human liver cancer cell Hep3B and human non-small cell lung cancer H23 (American Type Culture Collection ATCC) at 10⁴ cells/50 μ l per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;

(3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50 μ l per well, with the effect target ratio of 4:1 and Mock T cells transferred with empty pNB328 as the control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve;

[0212] The results are shown in Figure 26. The killing effect of EHCAR-EK-28TIZ-antiPD1 T cells that self-express PD1 antibody on a variety of tumor cells is significantly greater than those of EHCAR-EK-28TIZ T cells and control T cells.

Example 31: Comparison of cytokine release between EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ-antiPD1 T cells under specific stimulation of EGFR antigen

[0213] 96-well plates were coated overnight with 5ug/ml EGFR antigen at 4°C, washed 3 times with PBS, added with 1 $\times$ 10⁵ (100ul of volume) EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ-antiPD1 T cells prepared according to Example 2 and control Mock T cells (transferred with empty pNB328). Supernatant was collected after 24 hours of culture. The cytokine secretion of these three T cells after being stimulated by EGFR antigen was detected.

[0214] The results are shown in Figure 27. The secretions of IL-2 and IFN- γ from EHCAR-EK-28TIZ-antiPD1 T cells are significantly higher than those from EHCAR-EK-28TIZ T cells and Mock-T, indicating that self-expressing PD1 antibodies can promote the secretion of cytokines by CAR-T cells.

Example 32: Anti-tumor effect of EHCAR-EK-28TIZ T cells, EHCAR-EK-28TIZ-antiPD1-wt T cells and EHCAR-EK-28TIZ-antiPD1 T cells *in vivo*

[0215] Twenty NSG mice of 4-6 weeks were divided into 5 groups on average, with 4 mice of each group inoculated with liver cancer cell line HCCLM3-LUC for 1 $\times$ 10⁷ per mouse. 10 days after tumor being formed, each group was injected via tail vein with PBS (100ul PBS), Mock-T cells, EHCAR-EK-28TIZ T cells, EHCAR-EK-28TIZ-antiPD1-wt T cells and EHCAR-EK-28TIZ-antiPD1 T cells (1 $\times$ 10⁷/100ul of each), respectively. The changes of the tumor fluorescence in mice were observed and recorded.

[0216] The results show that PBS, Mock-T, EHCAR-EK-28TIZ-antiPD1-wt T cells have no therapeutic effect on the tumor model, EHCAR-EK-28TIZ T cells and EHCAR-EK-28TIZ-antiPD1 T cells have good anti-tumor effects, and EHCAR-EK-28TIZ-antiPD1 T cells are significantly better. The details are shown in Figure 28.

Example 33: Detection of genomic expression level of Muc1CAR in T cell genome after PBMCs cells from different patients being modified by Muc1CAR gene and PD-1 antibody gene

[0217] Extract the genomic DNA of the Mock T cells, Muc1CAR T cells and Muc1CAR-antiPD1 T cells obtained in Example 2 (method by kit) using the experimental procedure based on the attached instructions, determine DNA concentration of Mock T cells, Muc1CAR T cells and Muc1CAR-antiPD1 T cells, and detect the expression level of Muc1CAR genome by real-time fluorescence quantitative PCR method, with the reaction procedure of 50°C, 2min $\rightarrow$ 95°C, 10min $\rightarrow$ 95°C, 15s $\rightarrow$ 60°C, 1min, for 40 cycle. Generate the CT value of the Muc1CAR in genome and the CT value of Actin, and then calculate the absolute copy number content according to the corresponding formula.

[0218] It is found that through the PB transposase system, the Muc1CAR genome has been integrated into the T cell genome, as shown in Table 8 below:

Table 8

Donor	Type of T cell	Copy Number (within a single cell)
Patient 1	Mock T	0.00
	Muc1CAR T	19.04
	Muc1CAR-antiPD1 T	22.82

(continued)

Donor	Type of T cell	Copy Number (within a single cell)
Patient 2	Mock T	0.00
	Muc1CAR T	10.14
	Muc1CAR-antiPD1 T	12.58
Patient 3	Mock T	0.00
	Muc1CAR T	20.46
	Muc1CAR-antiPD1 T	22.33

Example 34: Detection of differences of activation phenotype and cytokine secretion between Mock T cells, Muc1CAR T cells and Muc1CAR-antiPD1 T cell by flow cytometry

[0219]

1. The suspended Mock T cells, Muc1CAR T cells and Muc1CAR-antiPD1 T cells transferred with pNB328-Muc1CAR and pS328-m279V vector were washed twice with PBS, centrifuged at 1200rpm for 5min, and added with 2ul of the isotype control antibody IgG1-PE, the fluorescent flow cytometry antibodies anti-CD25-PE, anti-LAG3-PE, anti-PD1-PE; isotype control antibody IgG1-PC5, the fluorescent flow cytometry antibody anti-CD107-PC5; isotype control antibody IgG1 FITC, fluorescent flow cytometry antibody anti-CD62L-FITC; isotype control antibody IgG1-PC5, fluorescent flow cytometry antibody anti-CD45RO-PC5; isotype control antibody IgG1-PE, fluorescent flow cytometry antibody anti-CCR7-PE, respectively. The precipitate was flicked to make it mix evenly, the cells were incubated at room temperature in the dark for 30 min, washed with PBS once, added with 400 μ l PBS and transferred to a flow tube, then analyzed on the machine.

The results show that the PD-1 single-chain antibody secreted by Muc1CAR-antiPD1 T cells can block the PD-1 protein on the surface of T cells well. Muc1CAR T cells and Muc1CAR-antiPD1 T cells have obvious killing activity *in vitro* and can also promote the formation of memory T, while activation marker CD25 is significantly higher than Mock T cells and depletion marker LAG3 of Muc1CAR-antiPD1 T cells is significantly lower than Mock T cells and Muc1CAR T cells. The details are shown in Figures 29A and 29B.

2. 1×10^6 Muc1CAR T cells and Muc1CAR-antiPD1 T cells transferred with pNB328-Muc1CAR and pS328-m279V vector obtained in Example 2 were added to 1.5ml EP tubes respectively, washed twice with PBS, centrifuged at 1200rpm for 5min, added with 2ul of α -CD3CD4CD8 antibody, incubated at room temperature in the dark for 30min, washed once with PBS, added with 400ul PBS and transferred to the flow tube, and assayed on the machine.

It is found that no big difference is shown in the percentages of CD3⁺CD4⁺ and CD3⁺CD8⁺ cells in Muc1CAR T cells, Muc1CAR-antiPD1 T cells and Mock T cells, as shown in 29C.

3. 24-well plates were coated overnight with 5ug/ml Muc1 antigen at 4°C, washed 3 times with PBS, added with 3×10^5 of Mock T cells, Muc1CAR T cells and Muc1CAR-antiPD1 T cells transferred with pNB328-Muc1CAR and pS328-m279V vector prepared according to Example 2. Supernatant was collected after 24 hours of culture. BD™CBA Human Th1/Th2 Cytokine Kit II was used to detect the secretion of cytokines of Muc1CAR T cells and Muc1CAR-antiPD1 T cells after stimulated by Muc1 antigen:

(1) Mix human IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ capture magnetic beads by vortex, add 50ul of mixed beads to each tube;

(2) Add 50ul of human Th1/Th2 cytokine standard (diluted to 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156pg/ml, 80pg/ml, 40pg/ml, 20pg/ml, or Opg/ml) and 50ul of the sample to be tested (diluted by 2-fold with the diluents);

(3) Add 50ul of human Th1/Th2-II-PE detection antibody to each tube;

(4) Incubate at room temperature in the dark for 3h;

(5) Add 1ml of washing buffer to each tube, centrifuge at 200g for 5min, and discard the supernatant;

(6) Add 300ul of washing buffer to each tube to resuspend the cells, and transfer the cells to a flow cytometry tube to detect the fluorescence value by a flow cytometer.

[0220] The results show that the IL-2, TNF- α and IFN- γ secretion of Muc1CAR T cells and Muc1CAR-antiPD1 T cells are greatly improved as compared to Mock T cells. The IL-4, IL-6 and IL-10 secretion of the three cells are not substantially different, as shown in Figure 29D.

Example 35: Killing assay of Mock T cells, Muc1CAR T cells, and Muc1CAR-antiPD1 T cells on the cultured tumor cells *in vitro*

[0221] Target cells and effector cells that match MHC class I were selected, and Real-time label-free cell analysis system (RTCA) was used to detect the killing effect of the cells *in vitro*. The steps are as follows:

(1) Zero adjustment: Add 50 μ l DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;

(2) Target cell plating: Plate cervical cancer cell Hela, liver cancer cell HCC-LM3 and lung cancer cell A549 (American Type Culture Collection ATCC) at 10⁴ cells/50 μ l per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;

(3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells (the Mock T cells, Muc1CAR T cells and Muc1CAR-antiPD1 T cells transferred with pNB328-Muc1CAR and pS328-m279V vector prepared according to Example 2) at 50 μ l per well, with the effect target ratio of 4:1 and Mock T cells without a transferred vector as the control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve.

[0222] The results show that Muc1CAR T cells expressing PD-1 antibody are superior to Muc1CAR T cells in killing all three tumor cells. The details are shown in Figure 30.

Example 36: *In vivo* function study of Muc1CAR T cells that express PD-1 antibody

[0223]

Step 1: 15 of 4-6 weeks old NSG completely immunodeficient mice, with an average weight of 22-27g, were provided by Beijing Vitalstar Biotech Co., Ltd., and raised by a SPF animal laboratory.

Step 2: Adhered human cervical cancer cell Hela in logarithmic growth phase cultured *in vitro* were digested with 0.25% trypsin, centrifuged, collected and resuspended in PBS solution. The cells were centrifuged at 3000g for 2 minutes at room temperature, the supernatant was discarded and the cells were resuspended in PBS solution, centrifuged and collected, and the concentration of the cell suspension was adjusted to 5 $\times$ 10⁷ cells/ml.

Step 3: The Hela-luc cells were inoculated subcutaneously in the dorsum of the right rib of the mouse at 0.1 ml/mouse. About 10 days after the inoculation, the size of the tumor was observed by an *in vivo* imager, and the NSG immunodeficient mice were randomly divided into 4 groups (five mice each). Each group was injected through the tail vein with PBS (100 μ l), Mock T cells, Muc1CAR T cells, Muc1CAR-antiPD1-wt T cells transferred with pNB328-Muc1CAR and pS328-m279V-wt, and Muc1CAR-antiPD1 T cells transferred with pNB328-Muc1CAR and pS328-m279V, prepared according to Example 2 (1 $\times$ 10⁷ cells/mouse).

Step 4: The living conditions of mice were observed every day and the change of the tumor in each mouse was observed by *in vivo* imager every 10 days.

[0224] The results are shown in Figure 31.

Figure 37: Detection of CD47 expression in Mock T cells, EGFR-CAR T cells, and α CD47-EGFR-CAR T cells.

[0225] The Mock T cells, EGFR-CAR T cells and α CD47-EGFR-CAR T cells prepared according to Example 2 were collected, and the expression of CD47 was detected using the flow cytometry antibody (BD) murine anti-human CD47-FITC by the flow cytometry as in Example 3.

[0226] The results are shown in Figure 32. The CD47 antibody secreted by α CD47-EGFR-CAR T cells can block the self-expressed CD47 of the cells.

Example 38: Killing effect of Mock T cells, EGFR-CAR T cells and α CD47-EGFR-CAR T cells on tumor cells

[0227] Three types of EGFR-positive cells were selected as target cells: lung cancer cell line H23, ovarian cancer cell line SKOV3, and pancreatic cancer cell line ASPC-1. Real-time label-free cell analysis system (RTCA, ACEA) was used to detect the *in vitro* killing effect of the Mock T cells, EGFR-CAR T cells and α CD47-EGFR-CAR T cells obtained in Example 2. The steps are as follows:

(1) Zero adjustment: Add 50 μ l DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;

(2) Target cell plating: Plate lung cancer cell line H23, ovarian cancer cell line SKOV3, pancreatic cancer cell line ASPC-1 (American Type Culture Collection ATCC) at 10^4 cells/50 μ l per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells; (3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50 μ l per well, with the effect target ratio of 4:1 and Mock T cells transferred with empty pNB328 as the control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve.

[0228] The results are shown in Figure 33. The *in vitro* killing effects of EGFR-CAR T cells and α CD47-EGFR-CAR T cells are significantly higher than that of Mock T cells, and the self-expression of CD47 antibody does not affect the killing effect of CAR-T cells.

Example 39: α CD47-EGFR-CAR T cell culture supernatant can block CD47 on the surface of tumor cells

[0229] The culture supernatants of α CD47-EGFR-CAR T cells obtained in Example 2 were co-cultured with lung cancer cell line H23, ovarian cancer cell line SKOV3, and pancreatic cancer cell line ASPC-1, respectively. After 24 hours, the tumor cells were collected to detect CD47 expression, compared with those without co-culture with α CD47-EGFR-CAR T cell supernatant. Flow cytometry is the same as in Example 3.

[0230] The results are shown in Figure 34. The CD47 antibody in the supernatant of the α CD47-EGFR-CAR T cells can block the CD47 on the surface of the tumor cells.

Example 40: Blocking CD47 on the surface of the tumor cells can enhance the phagocytosis of macrophages to the tumor cells

[0231]

1. Isolation and culture of macrophages: Ficoll density gradient centrifugation method was used to isolate peripheral blood mononuclear cells (PBMC), which were adherently cultured at 37°C in a 5% CO₂ incubator for 4 hours. The non-adherent cells were washed away by pre-warmed medium, and AIM-V medium and rhGM-CSF were added (final concentration of 1000U/ml). After two and a half days, the medium was exchanged and the cells were cultured for 7 days to obtain adherent cells, which are macrophages.

2. Phagocytosis of macrophages to tumor cells: the tumor cells were stained to blue with Hoechst dye, and the macrophages were stained to red with CM-Dil, by the staining methods according to the manufacturer's instructions. The two stained cells were mixed and divided to two parts: one was added with the culture supernatant of EGFR-CAR T cells prepared according to Example 2 as a control, and the other was added with the culture supernatant of α CD47-EGFR-CAR T cells prepared according to Example 2. Phagocytosis was observed using a confocal microscope, and the phagocytosis efficiency was counted.

[0232] The results are shown in Figure 35. The phagocytosis by macrophage for the α CD47-EGFR-CAR T cells culture supernatant group is significantly higher than that of the control group.

Example 41: Anti-tumor effect of α CD47-EGFR-CAR T cells *in vivo*

[0233] Twenty NSG mice of 4-6 weeks were divided into 5 groups on average, with 4 mice of each group inoculated with lung cancer cell line H23 for 1×10^7 per mouse. 10 days after tumor being formed, each group was injected via tail vein with PBS (100ul PBS), Mock T cells, EGFR-CAR T cells, wt- α CD47-EGFR-CAR T cells and α CD47-EGFR-CAR T cells obtained in Example 2 (1×10^7 /100ul of each), respectively. The tumor volume was observed and recorded. The results show that PBS, Mock T cells, wt- α CD47-EGFR-CAR T cells have no therapeutic effect on tumor models, EGFR-CAR T cells and α CD47-EGFR-CAR T cells have good anti-tumor effects, and α CD47-EGFR-CAR T cells are more effective. The details are shown in Figure 36.

Example 42: Detection of CD47 expression in Mock, Meso3CAR and α CD47-Meso3CAR T cells.

[0234] The Mock T cells, Meso3CAR T cells and α CD47-Meso3CAR T cells prepared according to Example 2 were collected, and the expression of PD1 was detected using the flow cytometry antibody FITC-murine anti-human CD47 (BD) by the flow cytometry method as in Example 3.

[0235] The results are shown in Figure 37. The CD47 antibody secreted by α CD47-Meso3CAR T cells can block the expression of CD47 on the surface of the cells.

Example 43: Killing effect of Mock, Meso3CAR and α CD47-Meso3CAR T cells on tumor cells

[0236] Three types of EGFR-positive cells were selected as target cells: gastric cancer cell line Hgc27, ovarian cancer cell line SKOV3, and pancreatic cancer cell line ASPC-1. Real-time label-free cell analysis system (RTCA, ACEA) was used to detect the *in vitro* killing effect of the Mock T cells, Meso3CAR T cells and α CD47-Meso3CAR T cells obtained in Example 2. The steps are as follows:

(1) Zero adjustment: Add 50 μ l DMEM or 1640 culture medium to each well, put it into the instrument, select step 1, and adjust zero;

(2) Target cell plating: Plate gastric cancer cell line Hgc27, ovarian cancer cell line SKOV3, pancreatic cancer cell line ASPC-1 (American Type Culture Collection ATCC) at 10⁴ cells/50 μ l per well on a plate containing detection electrodes, let rest for a few minutes to stabilize the cells, then put them into the instrument, start step 2 to culture the cells;

(3) Adding effector cells: After 24h culture of target cells, pause step 2 and add effector cells at 50 μ l per well, with the effect target ratio of 4:1 and Mock T cells transferred with empty pNB328 as the control, start step 3 to continue co-cultivation for 24h, then generate the cell proliferation curve.

[0237] The results are shown in Figure 38. The *in vitro* killing effects of Meso3CAR T cells and α CD47-Meso3CAR T cells are significantly higher than that of Mock T cells, and the expression of CD47 antibody does not affect the killing effect of CAR-T cells.

Example 44: α CD47-Meso3CAR T cell culture supernatant can block CD47 on the surface of tumor cells

[0238] The culture supernatants of α CD47-Meso3CAR T cells obtained in Example 2 were co-cultured with gastric cancer cell line Hgc27, ovarian cancer cell line SKOV3, and pancreatic cancer cell line ASPC-1, respectively. After 24 hours, the tumor cells were collected to detect CD47 expression, compared with those without co-culture with α CD47-Meso3CAR T cell supernatant. The flow cytometry method is the same as above.

[0239] The results are shown in Figure 39. The CD47 antibody in the supernatant of the α CD47-Meso3CAR T cells can block the expression of CD47 by the tumor cells.

Example 45: Blocking CD47 on the surface of the tumor cells can enhance the phagocytosis of macrophages to the tumor cells

[0240]

1. Isolation and culture of macrophages: Ficoll density gradient centrifugation method was used to isolate peripheral blood mononuclear cells (PBMC), which were adherently cultured at 37°C in a 5% CO₂ incubator for 4 hours. The non-adherent cells were washed away by pre-warmed medium, and AIM-V medium and rhGM-CSF were added (final concentration of 1000U/ml). After two and a half days, the medium was exchanged and the cells were cultured for 7 days to obtain adherent cells, which are macrophages.

2. Phagocytosis of macrophage to tumor cells: the tumor cells were stained to blue with Hoechst dye, and the macrophages were stained to red with CM-Dil, by the staining methods according to the manufacturer's instructions. The two stained cells were mixed and divided to two parts: one was added with the culture supernatant of Meso3CAR T cells prepared according to Example 2 as a control, and the other was added with the culture supernatant of α CD47-Meso3CAR T cells prepared according to Example 2. Phagocytosis was observed using a confocal microscope, and the phagocytosis efficiency was counted.

[0241] The results show that the phagocytosis by macrophage for the α CD47-Meso3CAR T cells culture supernatant group is significantly higher than that of the control group. The statistical results are shown in Figure 40.

Example 46: Anti-tumor effect of α CD47-Meso3CAR T cells *in vivo*

[0242] Twenty NSG mice of 4-6 weeks were divided into 5 groups on average, with 4 mice of each group inoculated with lung cancer cell line H23 for 1 $\times$ 10⁷ per mouse. 10 days after tumor being formed, each group was injected via tail vein with PBS (100 μ l PBS), Mock T cells, Meso3CAR T cells, wt- α CD47-Meso3CAR T cells and α CD47-Meso3CAR T cells obtained according to Example 2 (1 $\times$ 10⁷/mouse), respectively. The tumor volume was observed and recorded.

[0243] The results show that PBS, Mock T cells, wt- α CD47-Meso3CAR T cells have no therapeutic effect on the tumor models, while α CD47-Meso3CAR T cells have good anti-tumor effects.

[0244] The details are shown in Figure 41.

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Sequence Listing

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SHANGHAI CELL THERAPY GROUP CO., LTD

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EP 3 733 839 A1

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<223> Description of Artificial Sequence: amino acid sequence of mutant anti-PD-1 antibody

<400> 3
Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15
Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30
Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Gly
35 40 45
Val Ser Thr Ser Gly Tyr Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro
50 55 60
Gly Gln Ala Pro Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser
65 70 75 80
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95
Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
100 105 110
Gln His Ser Arg Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val
115 120 125
Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
130 135 140
Gly Ser Gln Val Gln Leu Val Gln Ser Gly Val Glu Val Lys Lys Pro
145 150 155 160
Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
165 170 175
Asn Tyr Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
180 185 190
Trp Met Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu
195 200 205
Lys Phe Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr
210 215 220
Ala Tyr Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr
225 230 235 240
Tyr Cys Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp
245 250 255
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Glu Ser Lys Tyr Gly Pro
260 265 270
Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val
275 280 285
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
290 295 300
Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
305 310 315 320
Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
325 330 335
Thr Lys Pro Arg Glu Glu Gln Phe Gln Ser Thr Tyr Arg Val Val Ser
340 345 350
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
355 360 365
Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
370 375 380
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
385 390 395 400

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Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
405 410 415
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
420 425 430
5 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
435 440 445
Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
450 455 460
Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
465 470 475 480
10 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
485 490 495

<210> 4
<211> 1488
15 <212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: nucleotide sequence of mutant
anti-PD-1 antibody

20 <400> 4
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60
gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 120
ctctcctgca gggccagcaa aggtgtcagt acatctggct atagttattt gacttggtat 180
25 caacagaaac ctggccaggc tcccaggctc ctcatctatc ttgcatccta cctagaatct 240
ggcgtcccag ccaggttcag tggtagtggg tctgggacag acttcactct caccatcagc 300
agcctagagc ctgaagattt tgcagtttat tactgtcagc acagcaggga ccttccgctc 360
acgttcggcg gagggaccaa agtgagatc aaaggtggag gcggttcagg cggaggtggc 420
agcggcggtg gcgggtcgca ggtgcagctg gtgcagtcgg gcgtggaggt gaagaagcct 480
ggcgccctccg tcaaggtgtc ctgtaaggcc tccggctaca ccttcaccaa ctactacatg 540
30 tactgggtgc ggcaggcccc aggccaggga ctggagtgga tgggcggcat caacccttcc 600
aacggcggca ccaacttcaa cgagaagttc aagaaccggg tgaccctgac caccgactcc 660
tccaccacaa ccgcctacat ggaactgaag tccctgcagt tcgacgacac cgccgtgtac 720
tactgcgcca ggcgggacta ccggttcgac atgggcttcg actactgggg ccagggcacc 780
accgtgaccg tgtcctccga gtccaaatat ggtcccccac gccaccatg cccagcacct 840
gagttcgagg ggggaccatc agtcttcctg ttcccccaa aacccaagga cactctcatg 900
35 atctcccgga cccctgaggt cacgtgcgtg gtggtggacg tgagccagga agaccccgag 960
gtccagttca actggtacgt ggatggcgtg gaggtgcata atgccaagac aaagccgcgg 1020
gaggagcagt tccagagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac 1080
tggtgaacg gcaaggagta caagtgaag gtctccaaca aaggcctccc gtcctccatc 1140
gagaaaacca tctccaaagc caaagggcag ccccgagagc cacaggtgta caccctgccc 1200
40 ccatcccagg aggagatgac caagaaccag gtcagcctga cctgcctggt caaaggcttc 1260
taccacagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag 1320
accacgcctc ccgtgctgga ctccgacggc tccttcttcc tctacagcag gctaaccgtg 1380
gacaagagca ggtggcagga ggggaatgtc ttctcatgct ccgtgatgca tgaggctctg 1440
cacaaccact acacacagaa gagcctctcc ctgtctctgg gtaaatga 1488

45 <210> 5
<211> 367
<212> PRT
<213> Artificial Sequence

50 <220>
<223> Description of Artificial Sequence: amino acid sequence of anti-CD47
antibody

55 <400> 5
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Asp Thr Thr Gly Glu Glu Glu Leu Gln Ile Ile Gln Pro Asp Lys Ser
20 25 30

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Val Leu Val Ala Ala Gly Glu Thr Ala Thr Leu Arg Cys Thr Ile Thr
35 40 45
Ser Leu Phe Pro Val Gly Pro Ile Gln Trp Phe Arg Gly Ala Gly Pro
50 55 60
5 Gly Arg Val Leu Ile Tyr Asn Gln Arg Gln Gly Pro Phe Pro Arg Val
65 70 75 80
Thr Thr Val Ser Asp Thr Thr Lys Arg Asn Asn Met Asp Phe Ser Ile
85 90 95
Arg Ile Gly Asn Ile Thr Pro Ala Asp Ala Gly Thr Tyr Tyr Cys Ile
100 105 110
10 Lys Phe Arg Lys Gly Ser Pro Asp Asp Val Glu Phe Lys Ser Gly Ala
115 120 125
Gly Thr Glu Leu Ser Val Arg Ala Lys Pro Glu Ser Lys Tyr Gly Pro
130 135 140
15 Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val
145 150 155 160
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
165 170 175
Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
180 185 190
20 Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
195 200 205
Thr Lys Pro Arg Glu Glu Gln Phe Gln Ser Thr Tyr Arg Val Val Ser
210 215 220
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
225 230 235 240
25 Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
245 250 255
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
260 265 270
Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
275 280 285
30 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
290 295 300
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
305 310 315 320
Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
325 330 335
35 Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
340 345 350
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
355 360 365

40 <210> 6
<211> 1104
<212> DNA
<213> Artificial Sequence

45 <220>
<223> Description of Artificial Sequence: nucleotide sequence of anti-CD47
antibody

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gaggaggagc tgcagatcat tcagcctgac aagtccgtgt tggttgcagc tggagagaca 120
50 gccactctgc gctgcactat cacctctctg ttccctgtgg ggcccatcca gtggttcaga 180
ggagctggac caggccgggt gttaatctac aatcaaagac agggccccctt ccccggtta 240
acaactgttt cagacaccac aaagagaaac aacatggact tttccatccg catcggtaac 300
atcacccag cagatgccgg cacctactac tgtatcaagt tccggaaagg gagccccgat 360
gacgtggagt ttaagtctgg agcaggcact gagctgtctg tgcgcgcca acccgagtc 420
55 aaatatggtc ccccatgccc accatgccca gcacctgagt tcgagggggg accatcagtc 480
ttcctgttcc ccccaaaacc caaggacact ctcatgatct cccggacccc tgaggtcacg 540
tgcgtggtgg tggacgtgag ccaggaagac cccgaggtcc agttcaactg gtacgtggat 600

	ggcgtggagg	tgcataatgc	caagacaaaag	ccgcggggagg	agcagttcca	gagcacgtac	660									
	cgtgtggtca	gcgtcctcac	cgtcctgcac	caggactggc	tgaacggcaa	ggagtacaag	720									
	tgcaaggtct	ccaacaaaag	cctcccgtcc	tccatcgaga	aaaccatctc	caaagccaaa	780									
	gggcagcccc	gagagccaca	ggtgtacacc	ctgcccccat	cccaggagga	gatgaccaag	840									
5	aaccagggtca	gcctgacctg	cctgggtcaaa	ggcttctacc	ccagcgacat	cgccgtggag	900									
	tgggagagca	atgggcagcc	ggagaacaac	tacaagacca	cgcctcccgt	gctggactcc	960									
	gacggctcct	tcttcctcta	cagcaggcta	accgtggaca	agagcaggtg	gcaggagggg	1020									
	aatgtcttct	catgctccgt	gatgcatgag	gctctgcaca	accactacac	acagaagagc	1080									
	ctctccctgt	ctctgggtaa	atga				1104									
10	<210>	7														
	<211>	486														
	<212>	PRT														
	<213>	Artificial Sequence														
	<220>															
15	<223>	Description of Artificial Sequence: amino acid sequence of CD19CAR														
	<400>	7														
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	1				5					10					15	
20	His	Ala	Ala	Arg	Pro	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu
			20						25					30		
	Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln
			35					40					45			
	Asp	Ile	Ser	Lys	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr
		50					55					60				
25	Val	Lys	Leu	Leu	Ile	Tyr	His	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro
		65				70					75				80	
	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile
				85						90				95		
	Ser	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly
				100					105					110		
30	Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Thr
			115					120					125			
	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Glu
		130					135					140				
	Val	Lys	Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Ala	Pro	Ser	Gln	Ser
35		145				150					155				160	
	Leu	Ser	Val	Thr	Cys	Thr	Val	Ser	Gly	Val	Ser	Leu	Pro	Asp	Tyr	Gly
				165						170				175		
	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Arg	Lys	Gly	Leu	Glu	Trp	Leu	Gly
			180						185					190		
40	Val	Ile	Trp	Gly	Ser	Glu	Thr	Thr	Tyr	Tyr	Asn	Ser	Ala	Leu	Lys	Ser
		195						200					205			
	Arg	Leu	Thr	Ile	Ile	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Leu	Lys
		210				215						220				
	Met	Asn	Ser	Leu	Gln	Thr	Asp	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	Lys
		225				230					235				240	
45	His	Tyr	Tyr	Tyr	Gly	Gly	Ser	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly
				245						250				255		
	Thr	Ser	Val	Thr	Val	Ser	Ser	Thr	Thr	Thr	Pro	Ala	Pro	Arg	Pro	Pro
			260						265					270		
	Thr	Pro	Ala	Pro	Thr	Ile	Ala	Ser	Gln	Pro	Leu	Ser	Leu	Arg	Pro	Glu
			275					280					285			
50	Ala	Cys	Arg	Pro	Ala	Ala	Gly	Gly	Ala	Val	His	Thr	Arg	Gly	Leu	Asp
		290					295					300				
	Phe	Ala	Cys	Asp	Ile	Tyr	Ile	Trp	Ala	Pro	Leu	Ala	Gly	Thr	Cys	Gly
		305				310					315				320	
	Val	Leu	Leu	Leu	Ser	Leu	Val	Ile	Thr	Leu	Tyr	Cys	Lys	Arg	Gly	Arg
				325						330				335		
55	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln
			340						345					350		

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Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
 355 360 365
 Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
 370 375 380
 5 Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
 385 390 395 400
 Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
 405 410 415
 Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
 420 425 430
 10 Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
 435 440 445
 Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
 450 455 460
 Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
 465 470 475 480
 15 Gln Ala Leu Pro Pro Arg
 485

 <210> 8
 <211> 1461
 20 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: nucleotide sequence of CD19CAR

 25 <400> 8
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 ccggacatcc agatgacaca gactacatcc tccctgtctg cctctctggg agacagagtc 120
 accatcagtt gcagggcaag tcaggacatt agtaaattatt taaattggta tcagcagaaa 180
 ccagatggaa ctgttaaact cctgatctac catacatcaa gattacactc aggagtccca 240
 30 tcaaggttca gtggcagtgg gtctggaaca gattattctc tcaccattag caacctggag 300
 caagaagata ttgccactta cttttgccaa cagggttaata cgcttccgta cacgttcgga 360
 ggggggacta agttggaaat aacagggtgga ggcgggttcag gcggaggtgg cagcggcggt 420
 ggcggttcgg aggtgaaact gcaggagtca ggacctggcc tgggtggcgcc ctcacagagc 480
 ctgtccgtca catgcactgt ctcaggggtc tcattaccgc actatggtgt aagctggatt 540
 cgccagcctc cacgaaaggg tctggagtgg ctgggagtaa tatggggtag tgaaaccaca 600
 35 tactataatt cagctctcaa atccagactg accatcatca aggacaactc caagagccaa 660
 gttttcttaa aaatgaacag tctgcaaact gatgacacag ccatttacta ctgtgccaaa 720
 cattattact acggtggtag ctatgctatg gactactggg gtcaaggaac ctcagtcacc 780
 gtctcctcaa ccacgacgcc agcgccgcga ccaccaacac cggcgcccac catcgctcg 840
 cagcccctgt ccctgcgccc agaggcgtgc cggccagcgg cggggggcgc agtgcacacg 900
 40 agggggctgg acttcgcctg tgatatctac atctgggcgc ccctggccgg gacttgtggg 960
 gtccttctcc tgtcactggg tatcaccctt tactgcaaac ggggcagaaa gaagtcctg 1020
 tatatatcca aacaaccatt tatgagacca gtacaaacta ctcaagagga agatggctgt 1080
 agctgccgat ttccagaaga agaagaagga ggatgtgaac tgagagtga gttcagcagg 1140
 agcgagacg cccccgcgta ccagcagggc cagaaccagc tctataacga gctcaatcta 1200
 ggacgaagag aggagtacga tgttttgac aagagacgtg gccgggaccc tgagatgggg 1260
 45 ggaaagccga gaaggaagaa ccctcaggaa ggcctgtaca atgaactgca gaaagataag 1320
 atggcggagg cctacagtga gattgggatg aaaggcgagc gccggagggg caaggggcac 1380
 gatggccttt accagggtct cagtacagcc accaaggaca cctacgacgc ctttcacatg 1440
 caggccctgc ccctcgctg a 1461

 <210> 9
 50 <211> 681
 <212> PRT
 <213> Artificial Sequence

 <220>
 55 <223> Description of Artificial Sequence: amino acid sequence of mesoCAR

 <400> 9

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	His	Ala	Ala	Arg	Pro	Ser	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly
				20					25					30		
5	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly
			35					40					45			
	Phe	Asp	Leu	Gly	Phe	Tyr	Phe	Tyr	Ala	Cys	Trp	Val	Arg	Gln	Ala	Pro
		50					55					60				
	Gly	Lys	Gly	Leu	Glu	Trp	Val	Ser	Cys	Ile	Tyr	Thr	Ala	Gly	Ser	Gly
	65					70					75					80
10	Ser	Thr	Tyr	Tyr	Ala	Ser	Trp	Ala	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg
					85					90					95	
	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala
				100					105					110		
	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Ser	Thr	Ala	Asn	Thr	Arg
			115					120					125			
15	Ser	Thr	Tyr	Tyr	Leu	Asn	Leu	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val
		130					135					140				
	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly
	145					150					155					160
	Ser	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val
					165					170						175
20	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Gln	Ala	Ser	Gln	Arg	Ile	Ser	Ser
				180					185					190		
	Tyr	Leu	Ser	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Val	Pro	Lys	Leu	Leu
		195						200					205			
	Ile	Tyr	Gly	Ala	Ser	Thr	Leu	Ala	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser
25		210					215					220				
	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln
	225					230					235					240
	Pro	Glu	Asp	Val	Ala	Thr	Tyr	Tyr	Cys	Gln	Ser	Tyr	Ala	Tyr	Phe	Asp
					245					250				255		
30	Ser	Asn	Asn	Trp	His	Ala	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
				260				265						270		
	Glu	Ser	Lys	Tyr	Gly	Pro	Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val
			275					280					285			
	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
		290					295					300				
35	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
	305					310					315					320
	Gln	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu
					325					330				335		
	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Gln	Ser	Thr
				340					345					350		
40	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn
			355					360					365			
	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser
		370					375					380				
	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln
45		385				390					395					400
	Val	Tyr	Thr	Leu	Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	
					405					410				415		
	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val
				420				425					430			
	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro
50			435					440					445			
	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr
		450					455					460				
	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val
					470						475					480
55	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu
					485					490					495	
	Ser	Leu	Gly	Lys	Pro	Phe	Trp	Val	Leu	Val	Val	Val	Gly	Gly	Val	Leu

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[illegible]

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agtgagattg ggatgaaagg cgagcgcccg aggggcaagg ggcacgatgg cctttaccag 1980
ggtctcagta cagccaccaa ggacacctac gacgcccttc acatgcaggc cctgccccct 2040
cgc 2043

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<210> 11
<211> 679
<212> PRT
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence: amino acid sequence of Meso3CAR

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<400> 11
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20 25 30
Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp
35 40 45
Leu Gly Phe Tyr Phe Tyr Ala Cys Trp Val Arg Gln Ala Pro Gly Lys
50 55 60
Gly Leu Glu Trp Val Ser Cys Ile Tyr Thr Ala Gly Ser Gly Ser Thr
65 70 75 80
Tyr Tyr Ala Ser Trp Ala Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95
Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100 105 110
Thr Ala Val Tyr Tyr Cys Ala Arg Ser Thr Ala Asn Thr Arg Ser Thr
115 120 125
Tyr Tyr Leu Asn Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
145 150 155 160
Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
165 170 175
Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Arg Ile Ser Ser Tyr Leu
180 185 190
Ser Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr
195 200 205
Gly Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
210 215 220
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
225 230 235 240
Asp Val Ala Thr Tyr Tyr Cys Gln Ser Tyr Ala Tyr Phe Asp Ser Asn
245 250 255
Asn Trp His Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Glu Ser
260 265 270
Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
275 280 285
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
290 295 300
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
305 310 315 320
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
325 330 335
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Gln Ser Thr Tyr Arg
340 345 350
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
355 360 365
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
370 375 380

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	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu	His	
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	aactacaaga	ccacgcctcc	cgtgctggac	tccgacggct	ccttcttctc	ctacagcagg	1380
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	tttattattt	tctgggtgag	gagtaagagg	agcaggctcc	tgacacagtga	ctacatgaac	1620
	atgactcccc	gccgccccgg	gccacccgc	aagcattacc	agccctatgc	cccaccacgc	1680
	gacttcgcag	cctatcgctc	cagagtgaag	ttcagcagga	gcgcagacgc	ccccgcgtac	1740
	cagcagggcc	agaaccagct	ctataacgag	ctcaatctag	gacgaagaga	ggagtacgat	1800
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	cctcaggaag	gctgtacaa	tgaactgcag	aaagataaga	tgccggaggc	ctacagtgag	1920
	attgggatga	aagggcagcg	ccggaggggc	aaggggcacg	atggccttta	ccagggtctc	1980
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	<213>	Artificial Sequence					
	<220>						
25	<223>	Description of Artificial Sequence: amino acid sequence of ErbBCAR					
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							30
	Ser	His	Asp	Gly	Tyr	Cys	Leu
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							40
							45
	Ala	Leu	Asp	Lys	Tyr	Ala	Cys
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							55
							60
35	Arg	Cys	Gln	Tyr	Arg	Asp	Leu
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	Ala	Lys	Glu	Ala	Ala	Ala	Lys
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							90
	Leu	Pro	Pro	Arg	Pro	Ala	Ala
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							100
							105
40	Gly	Pro	Ala	His	Pro	Val	Leu
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							115
							120
	Val	Ser	Ala	Phe	Tyr	Ser	Leu
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							135
	Val	Pro	Ile	Ser	Pro	Val	Ser
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							150
45	His	Val	Ala	Val	Asp	Leu	Ser
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							165
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							175
	Pro	Pro	Cys	Pro	Pro	Cys	Pro
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							185
							190
	Phe	Leu	Phe	Pro	Pro	Lys	Pro
							195
							200
							205
50	Pro	Glu	Val	Thr	Cys	Val	Val
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							215
							220
	Val	Gln	Phe	Asn	Trp	Tyr	Val
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							230
							235
	Thr	Lys	Pro	Arg	Glu	Gln	Phe
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55							250
							255
	Val	Leu	Thr	Val	Leu	His	Gln
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							265
							270

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Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
 275 280 285
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 290 295 300
 5 Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 305 310 315 320
 Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 325 330 335
 Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 340 345 350
 10 Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
 355 360 365
 Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Pro
 385 390 395 400
 15 Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
 405 410 415
 Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
 420 425 430
 Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
 435 440 445
 20 Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
 450 455 460
 Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
 465 470 475 480
 25 Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
 485 490 495
 Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
 500 505 510
 Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
 515 520 525
 30 Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
 530 535 540
 Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
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<210> 14

<211> 1740

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: nucleotide sequence of ErbBCAR

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 gatggtgtgt gcatgtatat tgaagcattg gacaagtatg catgcaactg tgttgttggt 180
 tacatcgggg agcgatgtca gtaccgagac ctgaagtggg gggaactgcg cgaagctgcc 240
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 50 cccgccgccg tgcccgtgcc cctgcgcgatg cagcccggcc ccgcccaccc cgtgctgagc 360
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 agccccacca gcgtgcccat cagccccgtg agcgtgggcc gcggccccga ccccgacgcc 480
 cagtgggccg tggacctgag ccgctacgag ggcgagtcga aatatggtcc cccatgccca 540
 ccatgccag cacctccgtg ggccggacca tcagtcttcc tgttcccccc aaaaccceaag 600
 gacactctca tgatctcccg gaccctgag gtcacgtgcg tggtggtgga cgtgagccag 660
 55 gaagaccccg aggtccagtt caactggtac gtggatggcg tggaggtgca taatgccaag 720
 acaaagccgc gggaggagca gttccagagc acgtaccgtg tggtcagcgt cctcaccgtc 780

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ctgcaccagg actggctgaa cggcaaggag tacaagtga aggtctccaa caaaggcctc      840
ccgtcctcca tcgagaaaac catctccaaa gccaaagggc agccccgaga gccacagggt      900
tacaccctgc ccccatccca ggaggagatg accaagaacc aggtcagcct gacctgcctg      960
gtcaaaggct tctacccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag     1020
5 aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctacagc     1080
aggctaaccg tggacaagag caggtggcag gaggggaatg tcttctcatg ctccgtgatg     1140
catgaggctc tgcacaacca ctacacacag aagagcctct ccctgtctct gggtaaaccc     1200
ttttgggtgc tgggtggtgt tgggtggagtc ctggcttgct atagcttgct agtaacagtg     1260
gcctttatta ttttctgggt gaggagtaag aggagcaggc tcctgcacag tgactacatg     1320
10 aacatgactc cccgccgcc cggggccacc cgcaagcatt accagcccta tgccccacca     1380
cgcgacttcg cagcctatcg ctccagagtg aagttcagca ggagcgcaga cgccccgcg      1440
taccagcagg gccagaacca gctctataac gagctcaatc taggacgaag agaggagtac     1500
gatgttttgg acaagagacg tggccgggac cctgagatgg ggggaaagcc gagaaggaag     1560
aaccctcagg aaggcctgta caatgaactg cagaaagata agatggcgga ggcctacagt     1620
gagattggga tgaaaggcga gcgccggagg ggcaaggggc acgatggcct ttaccagggt     1680
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<210> 15
<211> 678
<212> PRT
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence: amino acid sequence of MuclCAR

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Leu Gly Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser
35 35 40 45
30 Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln
50 55 60
Arg Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu
65 70 75 80
Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
85 90 95
35 Phe Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr
100 105 110
Tyr Cys Gln His Ser Arg Glu Leu Pro Phe Thr Phe Gly Gly Gly Thr
115 120 125
Lys Leu Glu Ile Lys Gly Gly Gly Ser Gly Gly Gly Ser Gly
40 130 135 140
Gly Gly Gly Ser Glu Val Gln Leu Glu Glu Ser Gly Gly Gly Leu Val
145 150 155 160
Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr
165 170 175
Phe Ser Gly Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg
45 180 185 190
Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Thr Tyr Ile Tyr Tyr
195 200 205
Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
210 215 220
50 Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala
225 230 235 240
Met Tyr Tyr Cys Ala Arg Leu Gly Gly Asp Asn Tyr Tyr Glu Tyr Phe
245 250 255
Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Glu Ser Lys
260 265 270
55 Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
275 280 285
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser

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	agtggtggtg	cttatatcta	ctatccagac	agtgtgaagg	ggcgattcac	catctccaga	660
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	tacaagacca	cgcctcccgt	gctggactcc	gacggctcct	tcttcctcta	cagcaggcta	1380
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	gctctgcaca	accactacac	acagaagagc	ctctccctgt	ctctgggtaa	acccttttgg	1500
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<211> 497

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: amino acid sequence of EGFR-CAR

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Leu Ser Val Ser Pro Gly Glu Arg Val Ser Phe Ser Cys Arg Ala Ser
35 40 45
Gln Ser Ile Gly Thr Asn Ile His Trp Tyr Gln Gln Arg Thr Asn Gly
50 55 60
Ser Pro Arg Leu Leu Ile Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile
65 70 75 80
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser
85 90 95
Ile Asn Ser Val Glu Ser Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln
100 105 110
Asn Asn Asn Trp Pro Thr Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
115 120 125
Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140
Gln Val Gln Leu Lys Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
145 150 155 160
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
165 170 175
Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu

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180 185 190
 Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
 195 200 205
 Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
 210 215 220
 Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
 225 230 235 240
 Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
 245 250 255
 Thr Leu Val Thr Val Ser Ser Phe Val Pro Val Phe Leu Pro Ala Lys
 260 265 270
 Pro Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile
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 Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala
 290 295 300
 Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr
 305 310 315 320
 Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu
 325 330 335
 Val Ile Thr Leu Tyr Cys Asn His Arg Ser Lys Arg Ser Arg Leu Leu
 340 345 350
 His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg
 355 360 365
 Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg
 370 375 380
 Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
 385 390 395 400
 Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
 405 410 415
 Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
 420 425 430
 Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
 435 440 445
 Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
 450 455 460
 Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
 465 470 475 480
 Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
 485 490 495
 Arg

<210> 18
 <211> 1494
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: nucleotide sequence of EGFR-CAR

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 gtcagtttct cctgcagggc cagtcagagt attggcacia acatacactg gtatcagcaa 180
 agaacaaatg gttctccaag gcttctcata aagtatgctt ctgagtctat ctctgggatc 240
 ccttccaggt ttagtgagcag tggatcaggg acagatttta ctcttagcat caacagtgtg 300
 gagtctgaag atattgcaga ttattactgt caacaaaata ataactggcc aaccacgttc 360
 ggtgctggga ccaagctgga gctgaaagggt ggaggcgggt caggcggagg tggcagcggc 420
 ggtggcgggt cgaggtgca gctgaagcag tcaggacctg gcctagtgca gccctcacag 480
 agcctgtcca tcacctgcac agtctctggt ttctcattaa ctaactatgg tgtacactgg 540
 gttcgccagt ctccaggaaa gggctctggag tggctgggag tgatatggag tgggtgaaac 600
 acagactata atacaccttt cacatccaga ctgagcatca acaaggacaa ttccaagagc 660
 caagttttct ttaaaatgaa cagtctgcaa tctaattgaca cagccatata ttactgtgcc 720

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agagccctca cctactatga ttacgagttt gcttactggg gccaaaggac tctgggtcact      780
gtctcttcgt tcgtgccggt ctctctgccca gcgaagccca ccacgacgcc agcgccgcga      840
ccaccaaacac cggcgcccac catcgcgctcg cagcccctgt ccctgcgccc agaggcgtgc      900
cggccagcgg cggggggcgc agtgacacacg aggggggctgg acttcgcctg tgatatctac      960
5 atctggggcg ccctggccgg gacttggtgg gtccttctcc tgtcactggt tatcaccctt     1020
tactgcaacc acaggagtaa gaggagcagg ctcttgacac gtgactacat gaacatgact     1080
ccccgcgcgc ccggggccac ccgcaagcat taccagccct atgccccacc acgcgacttc     1140
gcagcctatc gctccagagt gaagttcagc aggagcgagc acgccccgcg gtaccagcag     1200
ggccagaacc agctctataa cgagctcaat ctaggacgaa gagaggagta cgatgttttg     1260
10 gacaagagac gtggccggga ccctgagatg gggggaaagc cgagaaggaa gaaccctcag     1320
gaaggcctgt acaatgaact gcagaaagat aagatggcgg aggcctacag tgagattggg     1380
atgaaaggcg agcgccggag gggcaagggg cacgatggcc tttaccaggg tctcagtaca     1440
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<210> 19
15 <211> 78
<212> DNA
<213> Artificial Sequence

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<220>
20 <223> Description of Artificial Sequence: nucleotide sequence of Linker 2A

<400> 19
cgtaggaaac gaggcagcgg cgccacaaac ttctctctgc taaagcaagc aggtgatgtt      60
gaagaaaacc ccgggcct                                     78

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25 <210> 20
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30 <220>
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<400> 20
ccggcggggtt tctgacatcc ggcggggtttc tgacatccgg cgggtttctg acatccggcg      60
ggttttctgac atccggcggg tttctgacat ccggcggggtt tctgacatcc ggcggggtttc     120
35 tgacatccgg cgggtttctg acatccggcg gggtttctgac atccggcggg tgactcacia     180
ccccagaaac agacata                                     197

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40 <210> 21
<211> 94
<212> PRT
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence: amino acid sequence of mesothelin
Region I

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45 <400> 21
Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys Ala Arg Glu Ile
1          5          10          15
Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val
20          25          30
50 Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
35          40          45
Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu
50          55          60
Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu
55 65          70          75          80
Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val
85          90

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<210> 22
 <211> 111
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: amino acid sequence of mesothelin Region III

<400> 22

Tyr Pro Lys Ala Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr
 1 5 10 15
 Phe Val Lys Ile Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu
 20 25 30
 Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met
 35 40 45
 Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln
 50 55 60
 Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His
 65 70 75 80
 Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp
 85 90 95
 Thr Leu Gly Leu Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu
 100 105 110

<210> 23
 <211> 240
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: amino acid sequence of anti-mesothelin Region I scFv

<400> 23

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Glu Lys Pro Gly Ala
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 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
 20 25 30
 Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
 35 40 45
 Gly Leu Ile Thr Pro Tyr Asn Gly Ala Ser Ser Tyr Asn Gln Lys Phe
 50 55 60
 Arg Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Asp Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95
 Ala Arg Gly Gly Tyr Asp Gly Arg Gly Phe Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 115 120 125
 Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile
 130 135 140
 Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
 145 150 155 160
 Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser
 165 170 175
 Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
 180 185 190
 Gly Arg Phe Ser Gly Ser Gly Ser Gly Asn Ser Tyr Ser Leu Thr Ile
 195 200 205
 Ser Ser Val Glu Ala Glu Asp Asp Ala Thr Tyr Tyr Cys Gln Gln Trp
 210 215 220

Ser Lys His Pro Leu Thr Tyr Gly Ala Gly Thr Lys Leu Glu Ile Lys
 225 230 235 240

<210> 24
 <211> 45
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: amino acid sequence of Muc1
 membrane-proximal end

<400> 24

Gly Thr Ile Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr Lys
 1 5 10 15
 Thr Glu Ala Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser Val
 20 25 30
 Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala
 35 40 45

<210> 25
 <211> 229
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: amino acid sequence of wild type
 IgG4Fc

<400> 25

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe
 1 5 10 15
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 20 25 30
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 35 40 45
 Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
 50 55 60
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
 65 70 75 80
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 85 90 95
 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser
 100 105 110
 Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 115 120 125
 Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
 130 135 140
 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 145 150 155 160
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 165 170 175
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
 180 185 190
 Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser
 195 200 205
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 210 215 220
 Leu Ser Leu Gly Lys
 225

Claims

1. A T cell expressing an antibody or comprising a coding sequence of the antibody or an expression vector thereof, the antibody comprising an optional signal peptide, an antigen binding sequence, and a mutant Fc segment, wherein, the mutant Fc segment is a mutant Fc segment in which the amino acid residues at positions corresponding to positions 17 and 79 of the IgG4 Fc segment shown as SEQ ID NO: 25 are mutated to E and Q, respectively; preferably, the mutant Fc segment is a mutant IgG4 Fc segment, and amino acid sequence thereof is preferably as shown in amino acid residues 269-497 of SEQ ID NO: 1, preferably coding sequence thereof is as shown in nucleotide residues 805-1491 of SEQ ID NO: 2; preferably, an expression cassette of the antibody is integrated into the genome of the T cell.
2. The T cell according to claim 1, wherein, the signal peptide is a light chain signal peptide, and amino acid sequence thereof is preferably as shown in amino acid residues 1-20 of SEQ ID NO: 1, preferably coding sequence thereof is as shown in nucleotide residues 1-60 of SEQ ID NO: 2; and/or the antigen binding sequence is derived from an antibody or an antigen-binding fragment thereof that specifically binds to the antigen, such as a single-chain antibody, or from a ligand of a protein that functions in a tumor micro-environment or a fragment thereof that binds to the protein; preferably, the antibody is an agonistic antibody or an inhibitory antibody; preferably, the agonistic antibody is selected from antibodies directed against one or more of the following antigens: CD28, CD137, CD134, CD40, CD40L, ICOS, HVEM, CD2, CD27, CD30, GITR, LIGHT, DR3, SLAM, CD226, CD80 and CD86; preferably, the inhibitory antibody is selected from antibodies directed against one or more of the following antigens: PD-1, CTLA4, PDL1, PDL2, PDL3, TIM3, LAG3, CD47, BTLA, TIGIT, CD160, LAIR1, B7-H1, B7-1, VSIR and CD244; preferably, the ligand is a ligand of CD47.
3. The T cell according to claim 2, wherein, the agonistic antibody is a CD40 single-chain antibody; preferably, the amino acid sequence of the light chain variable region of the CD40 single-chain antibody is as shown in amino acid residues 21-146 of SEQ ID NO: 1, and/or the amino acid sequence of the heavy chain variable region of the CD40 single-chain antibody is as shown in amino acid residues 161-268 of SEQ ID NO: 1; preferably, the amino acid sequence of the CD40 single-chain antibody is as shown in amino acid residues 21-268 of SEQ ID NO: 1; the inhibitory antibody is a PD-1 single-chain antibody; preferably, the amino acid sequence of the light chain variable region of the PD-1 single-chain antibody is as shown in amino acid residues 21-131 of SEQ ID NO: 3, and/or the amino acid sequence of the heavy chain variable region of the PD-1 single-chain antibody is as shown in amino acid residues 147-266 of SEQ ID NO: 3; preferably, the amino acid sequence of the PD-1 single-chain antibody is as shown in amino acid residues 21-266 of SEQ ID NO: 3; the amino acid sequence of the CD47 ligand is as shown in amino acid residues 21-138 of SEQ ID NO: 5.
4. The T cell according to claim 3, wherein, the coding sequence of the light chain variable region of the CD40 single-chain antibody is as shown in nucleotide residues 60-438 of SEQ ID NO: 2, and/or the coding sequence of the heavy chain variable region thereof may be as shown in nucleotide residues 481-804 of SEQ ID NO: 2; preferably, the coding sequence of the CD40 single-chain antibody is shown as nucleotide residues 60-804 of SEQ ID NO: 2; the coding sequence of the light chain variable region of the PD-1 single-chain antibody is as shown in nucleotide residues 60-393 of SEQ ID NO: 4, and/or the coding sequence of the heavy chain variable region thereof is as shown in nucleotide residues 439-798 of SEQ ID NO: 4; preferably, the coding sequence of the PD-1 single-chain antibody is shown as nucleotide residues 60-798 of SEQ ID NO: 4; the coding sequence of the CD47 ligand may be as shown in nucleotide residues 60-414 of SEQ ID NO: 6.
5. The T cell according to claim 1, wherein the antibody is a CD40 antibody, a PD-1 antibody or a CD47 antibody; wherein, the amino acid sequence of the CD40 antibody is as shown in amino acid residues 21-497 of SEQ ID NO: 1, or as shown in SEQ ID NO: 1, the amino acid sequence of the PD-1 antibody is as shown in amino acid residues 21-495 of SEQ ID NO: 3, or as shown in SEQ ID NO: 3, the amino acid sequence of the CD47 antibody is as shown in amino acid residues 21-367 of SEQ ID NO: 5, or as shown in SEQ ID NO: 5; or wherein, the coding sequence of the antibody is as shown in nucleotide residues 60-1491 of SEQ ID NO: 2, or as shown in SEQ ID NO: 2; or is as shown in nucleotide residues 60-1485 of SEQ ID NO: 4, or as shown in SEQ ID NO: 4; or is as shown in nucleotide residues 60-1104 of SEQ ID NO: 6, or as shown in SEQ ID NO: 6.
6. The T cell according to any one of claims 1 to 5, wherein, the T cell is a CAR-T cell expressing a chimeric antigen

receptor, wherein the expression cassette of the antibody and the expression cassette of the chimeric antigen receptor are integrated into the genome of the T cell.

7. The T cell according to claim 6, wherein, the chimeric antigen receptor recognizes, targets, or specifically binds to one or more of the following antigens: CD19, CD20, CEA, GD2, FR, PSMA, PMEL, CA9, CD171/L1-CAM, IL-13R α 2, MART-1, ERBB2, NY-ESO-1, MAGE family proteins, BAGE family proteins, GAGE family proteins, AFP, MUC1, CD22, CD23, CD30, CD33, CD44v7/8, CD70, VEGFR1, VEGFR2, IL-11R α , EGP-2, EGP-40, FBP, GD3, PSCA, FSA, PSA, HMGA2, fetal acetylcholine receptor, LeY, EpCAM, MSLN, IGFR1, EGFR, EGFRvIII, ERBB3, ERBB4, CA125, CA15-3, CA19-9, CA72-4, CA242, CA50, CYFRA21-1, SCC, AFU, EBV-VCA, POA, β 2-MG and PROGRP; preferably the chimeric antigen receptor is those recognize, target, or specifically bind to CD19, mesothelin, EGFR, mucin or ErbB receptor family.
8. The T cell according to claim 7, wherein, the chimeric antigen receptor contains an optional signal peptide sequence, an antigen recognition region, a hinge region, a transmembrane region, an intracellular co-stimulatory signal domain and an intracellular signal domain; wherein, the signal peptide is selected from the group consisting of a CD8 signal peptide, a CD28 signal peptide, a CD4 signal peptide and a light chain signal peptide; the antigen recognition region is an amino acid sequence that recognizes, targets or specifically binds to the antigen of interest; the hinge region is selected from the group consisting of an extracellular hinge region of CD8, a hinge region of IgG1 Fc CH2CH3, an IgD hinge region, an extracellular hinge region of CD28, a hinge region of IgG4 Fc CH2CH3 and an extracellular hinge region of CD4, preferably the hinge region is 50 amino acid residues or more in length, more preferably 80 amino acid residues or more in length; preferably, the hinge region is a CD8 α hinge region or a hinge region of IgG4 Fc CH2CH3; the transmembrane region is selected from the group consisting of a transmembrane region of CD28, a transmembrane region of CD8, a transmembrane region of CD3 ζ , a transmembrane region of CD134, a transmembrane region of CD137, a transmembrane region of ICOS and a transmembrane region of DAP10; preferably is a transmembrane region of CD8 or a transmembrane region of CD28; the intracellular co-stimulatory signal domain is the intracellular domain of a co-stimulatory signal molecule, which is selected from the group consisting of intracellular domains of CD28, CD134/OX40, CD137/4-1BB, a lymphocyte-specific protein tyrosine kinase, an inducible T cell co-stimulatory factor and a DNAX activating protein 10, preferably is an intracellular domain of CD137/4-1BB or an intracellular domain of CD28; and/or the intracellular signal domain is an intracellular signal domain of CD3 ζ or an intracellular signal domain of Fc ϵ R1 γ , preferably an intracellular signal domain of CD3 ζ .
9. The T cell according to claim 8, wherein, the amino acid sequence of the signal peptide is as shown in amino acid residues 1-21 of SEQ ID NO: 7, or amino acid residues 1-22 of SEQ ID NO: 9, or amino acid residues 1-20 of SEQ ID NO: 11; the antigen recognition region is a single-chain antibody that recognizes, targets, or specifically binds to CD19, mesothelin, EGFR, or mucin, or consists of an amino acid sequence that recognizes, targets, or specifically binds to ErbB receptor family; the amino acid sequence of the hinge region is as shown in amino acid residues 264-308 of SEQ ID NO: 7, or amino acid residues 273-500 of SEQ ID NO: 9, or amino acid residues 264-318 of SEQ ID NO: 17; the amino acid sequence of the transmembrane region is as shown in amino acid residues 309-332 of SEQ ID NO: 7, or amino acid residues 501-528 of SEQ ID NO: 9, or amino acid residues 319-344 of SEQ ID NO: 17; the amino acid sequence of the intracellular co-stimulatory signal domain is as shown in amino acid residues 333-374 of SEQ ID NO: 7, or amino acid residues 529-569 of SEQ ID NO: 9; and/or the amino acid sequence of the intracellular signal domain is as shown in amino acid residues 375-486 of SEQ ID NO: 7.
10. The T cell according to claim 9, wherein, the coding sequence of the signal peptide is as shown in the nucleotide residues 1-63 of SEQ ID NO: 8, or the nucleotide residues 1-66 of SEQ ID NO: 10, or the nucleotide residues 1-60 of SEQ ID NO: 12; the coding sequence of the hinge region is as shown in nucleotide residues 790-924 of SEQ ID NO: 8, or nucleotide residues 817-1500 of SEQ ID NO: 10, or nucleotide residues 790-954 of SEQ ID NO: 18; the coding sequence of the transmembrane region is as shown in the nucleotide residues 925-996 of SEQ ID NO: 8, or the nucleotide residues 1501-1584 of SEQ ID NO: 10, or the nucleotide residues 955-1032 of SEQ ID NO: 18; the coding sequence of the intracellular co-stimulatory signal domain is as shown in the nucleotide residues 997-1122

of SEQ ID NO: 8, or the nucleotide residues 1585-1707 of SEQ ID NO: 10; and/or the coding sequence of the intracellular signal domain is as shown in the nucleotide residues 1123-1458 of SEQ ID NO: 8.

- 5 11. The T cell according to any of claims 9-10, wherein,
the amino acid sequence of the light chain variable region of the single-chain antibody that recognizes, targets or specifically binds to CD19 may be as shown in amino acid residues 22-128 of SEQ ID NO: 7, and/or the amino acid sequence of the heavy chain variable region thereof may be as shown in amino acid residues 144-263 of SEQ ID NO: 7; preferably, the amino acid sequence of the single-chain antibody is as shown in amino acid residues 22-263 of SEQ ID NO: 7;
- 10 the single-chain antibody that recognizes, targets or specifically binds to mesothelin antigen is a single-chain antibody directed against Region I or III of mesothelin, preferably the single-chain antibody directed against Region III of mesothelin; preferably, the amino acid sequence of the light chain variable region of the anti-mesothelin Region III single-chain antibody is as shown in amino acid residues 23-146 of SEQ ID NO: 9, and/or the amino acid sequence of the heavy chain variable region of the anti-mesothelin Region III single-chain antibody is as shown in amino acid residues 162-272 of SEQ ID NO: 9; preferably, the amino acid sequence of the single chain antibody that recognizes, targets or specifically binds to mesothelin antigen is as shown in amino acid residues 23-272 of SEQ ID NO: 9;
- 15 the antigen recognition region that recognizes, targets or specifically binds to the ErbB receptor family contains a fusion protein of natural TIE and Herin; wherein, the TIE consists of 7 amino acids at the N-terminus of human transcription growth factor α (TGF α) and 48 amino acids at the C-terminus of epidermal growth factor (EGF), preferably, the amino acid sequence of TIE is as shown in amino acid residues 23-77 of SEQ ID NO: 13; the Herin is the 79 amino acids encoded by intron 8 in Herstatin, preferably, the amino acid sequence of Herin is as shown in amino acid residues 93-171 of SEQ ID NO: 13; preferably, the antigen recognition region is as shown in amino acid residues 23-171 of SEQ ID NO: 13;
- 20 the amino acid sequence of the light chain variable region and the amino acid sequence of the heavy chain variable region of the single-chain antibody that recognizes, targets or specifically binds to mucin antigen are derived from an antibody against the amino acid sequence of the membrane-proximal end of Muc1, preferably, the amino acid sequence of the membrane-proximal end of Muc1 is as shown in SEQ ID NO: 24; preferably, the amino acid sequence of the light chain variable region of the single chain antibody is as shown in amino acid residues 23-133 of SEQ ID NO: 15, and/or the amino acid sequence of the heavy chain variable region is as shown in amino acid residues 149-269 of SEQ ID NO: 15; preferably, the amino acid sequence of the single chain antibody is as shown in amino acid residues 23-269 of SEQ ID NO: 15;
- 25 the antigen recognition region that recognizes, targets, or specifically binds to EGFR is a single chain antibody formed by the light chain variable region and the heavy chain variable region of an antibody specific for EGFR; preferably, the amino acid sequence of the light chain variable region of the single-chain antibody is as shown in amino acid residues 23-129 of SEQ ID NO: 17, and/or the amino acid sequence of the heavy chain variable region is as shown in amino acid residues 145-263 of SEQ ID NO: 17; preferably, the amino acid sequence of the single chain antibody is as shown in amino acid residues 23-263 of SEQ ID NO: 17.
- 30 12. The T cell according to claim 11, wherein, the chimeric antigen receptor contains, in the order from the N-terminus to the C-terminus, an optional signal peptide sequence, an antigen recognition region, the hinge region of CD8 α or the hinge region of IgG4 CH2CH3, the transmembrane region of CD8 or the transmembrane region of CD28, the intracellular domain of 4-1BB or CD28, and the intracellular signal domain of CD3 ζ ;
- 35 preferably, the chimeric antigen receptor is selected from the group consisting of:
- 40 (1) a chimeric antigen receptor targeting CD19, with the amino acid sequence thereof being as shown in amino acid residues 22-486 of SEQ ID NO: 7, or as shown in SEQ ID NO: 7, and the coding sequence thereof being preferably as shown in nucleotide residues 64-1458 of SEQ ID NO: 8, or as shown in SEQ ID NO: 8;
- 45 (2) a chimeric antigen receptor targeting mesothelin, with the amino acid sequence thereof being as shown in amino acid residues 23-681 of SEQ ID NO: 9, or as shown in SEQ ID NO: 9, the coding sequence thereof being preferably as shown in nucleotide residues 67-2043 of SEQ ID NO: 10, or as shown in SEQ ID NO: 10, or the amino acid sequence thereof being as shown in amino acid residues 21-679 of SEQ ID NO: 11, or as shown in SEQ ID NO: 11, the coding sequence thereof being preferably as shown in nucleotide residues 61-2037 of SEQ ID NO: 12, or as shown in SEQ ID NO: 12;
- 50 (3) a chimeric antigen receptor targeting ErbB family, with the amino acid sequence thereof being as shown in amino acid residues 23-580 of SEQ ID NO: 13, or as shown in SEQ ID NO: 13, and the coding sequence thereof being preferably as shown in nucleotide residues 67-1740 of SEQ ID NO: 14, or as shown in SEQ ID NO: 14;
- 55 (4) a chimeric antigen receptor targeting mucin, with the amino acid sequence thereof being as shown in amino

acid residues 23-678 of SEQ ID NO: 15, or as shown in SEQ ID NO: 15, and the coding sequence thereof being preferably as shown in nucleotide residues 67-2034 of SEQ ID NO: 16, or as shown in SEQ ID NO: 16; and
 (5) a chimeric antigen receptor targeting EGFR, with the amino acid sequence thereof being as shown in amino acid residues 23-497 of SEQ ID NO: 17, or as shown in SEQ ID NO: 17, and the coding sequence thereof being preferably as shown in nucleotide residues 67-1491 of SEQ ID NO: 18, or as shown in SEQ ID NO: 18.

13. An antibody, comprising an optional signal peptide, an antigen binding sequence, and a mutant Fc segment, wherein, the mutant Fc segment is a mutant Fc segment in which the amino acid residues at positions corresponding to positions 17 and 79 of the IgG4 Fc segment shown as SEQ ID NO: 25 are mutated to E and Q, respectively; preferably, the mutant Fc segment is a mutant IgG4 Fc segment, and amino acid sequence thereof is preferably as shown in amino acid residues 269-497 of SEQ ID NO: 1, preferably coding sequence thereof is as shown in nucleotide residues 805-1491 of SEQ ID NO: 2.

14. The antibody according to claim 13, wherein, the signal peptide is a light chain signal peptide, and amino acid sequence thereof is preferably as shown in amino acid residues 1-20 of SEQ ID NO: 1, preferably coding sequence thereof is as shown in nucleotide residues 1-60 of SEQ ID NO: 2; the antigen binding sequence is derived from an antibody or an antigen-binding fragment thereof that specifically binds to the antigen, such as a single-chain antibody, or from a ligand of a protein that functions in the tumor microenvironment or a fragment thereof that binds to the protein; preferably, the antibody is an agonistic antibody or an inhibitory antibody; preferably, the agonistic antibody is selected from antibodies directed against one or more of the following antigens: CD28, CD137, CD134, CD40, CD40L, ICOS, HVEM, CD2, CD27, CD30, GITR, LIGHT, DR3, SLAM, CD226, CD80 and CD86; preferably, the inhibitory antibody is selected from antibodies directed against one or more of the following antigens: PD-1, CTLA4, PDL1, PDL2, PDL3, TIM3, LAG3, CD47, BTLA, TIGIT, CD160, LAIR1, B7-H1, B7-1, VSIR and CD244; preferably, the ligand is a ligand of CD47.

15. The antibody according to claim 14, wherein, the agonistic antibody is a CD40 single-chain antibody; preferably, the amino acid sequence of the light chain variable region of the CD40 single-chain antibody is as shown in amino acid residues 21-146 of SEQ ID NO: 1, and/or the amino acid sequence of the heavy chain variable region of the CD40 single-chain antibody is as shown in amino acid residues 161-268 of SEQ ID NO: 1; preferably, the amino acid sequence of the CD40 single-chain antibody is as shown in amino acid residues 21-268 of SEQ ID NO: 1; the inhibitory antibody is a PD-1 single-chain antibody; preferably, the amino acid sequence of the light chain variable region of the PD-1 single-chain antibody is as shown in amino acid residues 21-131 of SEQ ID NO: 3, and/or the amino acid sequence of the heavy chain variable region of the PD-1 single-chain antibody is as shown in amino acid residues 147-266 of SEQ ID NO: 3; preferably, the amino acid sequence of the PD-1 single-chain antibody is as shown in amino acid residues 21-266 of SEQ ID NO: 3; the amino acid sequence of the CD47 ligand is as shown in amino acid residues 21-138 of SEQ ID NO: 5.

16. The antibody according to claim 15, wherein, the coding sequence of the light chain variable region of the CD40 single-chain antibody is as shown in nucleotide residues 60-438 of SEQ ID NO: 2, and/or the coding sequence of the heavy chain variable region thereof may be as shown in nucleotide residues 481-804 of SEQ ID NO: 2; preferably, the coding sequence of the CD40 single chain antibody is shown as nucleotide residues 60-804 of SEQ ID NO: 2; the coding sequence of the light chain variable region of the PD-1 single-chain antibody is as shown in nucleotide residues 60-393 of SEQ ID NO: 4, and/or the coding sequence of the heavy chain variable region thereof is as shown in nucleotide residues 439-798 of SEQ ID NO: 4; preferably, the coding sequence of the PD-1 single chain antibody is shown as nucleotide residues 60-798 of SEQ ID NO: 4; the coding sequence of the CD47 ligand may be as shown in nucleotide residues 60-414 of SEQ ID NO: 6.

17. The antibody according to claim 14, wherein the antibody is a CD40 antibody, a PD-1 antibody or a CD47 antibody; wherein, the amino acid sequence of the CD40 antibody is as shown in amino acid residues 21-497 of SEQ ID NO: 1, or as shown in SEQ ID NO: 1, the amino acid sequence of the PD-1 antibody is as shown in amino acid residues 21-495 of SEQ ID NO: 3, or as shown in SEQ ID NO: 3, the amino acid sequence of the CD47 antibody is as shown in amino acid residues 21-367 of SEQ ID NO: 5, or as shown in SEQ ID NO: 5; or wherein, the coding sequence of the antibody is as shown in nucleotide residues 60-1491 of SEQ ID NO: 2, or as shown in SEQ ID NO: 2; or is as shown in nucleotide residues 60-1485 of SEQ ID NO: 4, or as shown in SEQ ID NO: 4; or is as shown in nucleotide residues 60-1104 of SEQ ID NO: 6, or as shown in SEQ ID NO: 6.

18. A nucleic acid sequence selected from the coding sequence of the antibody according to any one of claims 13 to 17, or a complementary sequence thereof.

19. A nucleic acid construct comprising the nucleic acid sequence according to claim 18;
preferably, the nucleic acid construct being an expression cassette or a vector.

20. The nucleic acid construct according to claim 19, wherein, the nucleic acid construct is an expression vector or an integration vector for incorporating the expression cassette into the genome of a host cell;
preferably, the integration vector is an integration vector comprising a promoter, the coding sequence of the antibody according to any one of claims 13 to 17, and a polyA tailing signal sequence, in operable linkage between 5'LTR and 3'LTR, and not comprising a transposase coding sequence.

21. A composition, wherein the composition comprises the vector according to claim 20 and an optional transfection reagent;
preferably, the composition comprises the integration vector according to claim 20 and an integration vector for incorporating an expression cassette of a chimeric antigen receptor into the genome of a host cell; preferably, the chimeric antigen receptor is as defined in any of claims 6-12.

22. The composition according to claim 21, wherein the mass ratio of the integration vector for incorporating an expression cassette of a chimeric antigen receptor into the genome of a host cell to the integration vector according to claim 20 is 1-7:1-7, such as 1-5:1-5, preferably 1-3:1-3, more preferably 1-2:1-2, and even more preferably 1-2:1.

23. A kit, wherein the kit comprises the vector according to claim 20 and an optional transfection reagent;
preferably, the kit comprises the integration vector according to claim 20 and an integration vector for incorporating an expression cassette of a chimeric antigen receptor into the genome of a host cell; preferably, the chimeric antigen receptor is as defined in any of claims 6-12;
preferably, the kit contains the composition according to claim 21 or 22.

24. A pharmaceutical composition, comprising the T cell according to any one of claims 1-12 or comprising the T cell and the antibody described herein expressed by the T cell.

25. A host cell, comprising the nucleic acid sequence according to claim 18 or the nucleic acid construct according to any one of claims 19-20.

26. Use of the T cell according to any one of claims 1-12, the antibody according to any one of claims 13-17, the nucleic acid sequence according to claim 18, the nucleic acid construct according to any one of claims 19-20, and the host cell according to claim 25 in the preparation of a medicament for treating or preventing malignant tumors.

27. The use according to claim 26, wherein, the malignant tumor is selected from the group consisting of: acute B-lymphocytic leukemia, chronic B-lymphocytic leukemia, mantle cell lymphoma, non-Hodgkin's lymphoma, and multiple myeloma; or is a malignant tumor in which a cancer cell abnormally expresses mesothelin, at least one EGFR family member protein, a Mucl antigen, EGFR and/or CD47 on the cell surface; or is a malignant tumor mediated by CD40 or PD1.

28. A method for preparing the T cell according to any one of claims 6-12, the method comprises a step of transfecting the T cell with the following vectors:

- (1) the vector that is for transferring the expression cassette of the chimeric antigen receptor into the genome of the T cell and contains a transposase coding sequence, and
- (2) the vector that is for transferring the expression cassette of the antibody into the genome of the T cell and does not contain a transposase coding sequence;

preferably, the mass ratio of the vectors of (1) to (2) is 1-7:1-7, such as 1-5:1-5, preferably 1-3:1-3, more preferably 1-2: 1-2, more preferably 1-2:1.

Cytokine secretion

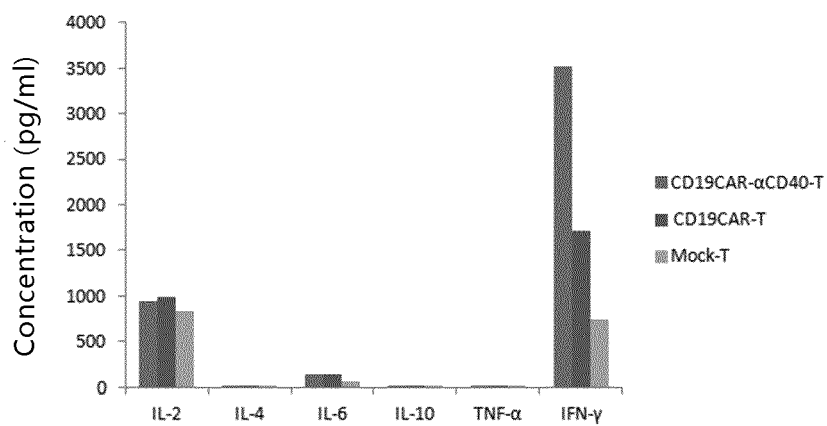


Figure 1

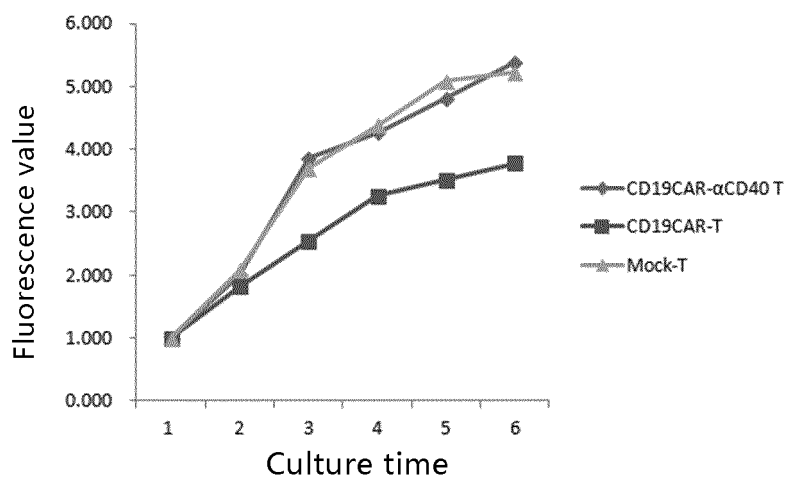


Figure 2

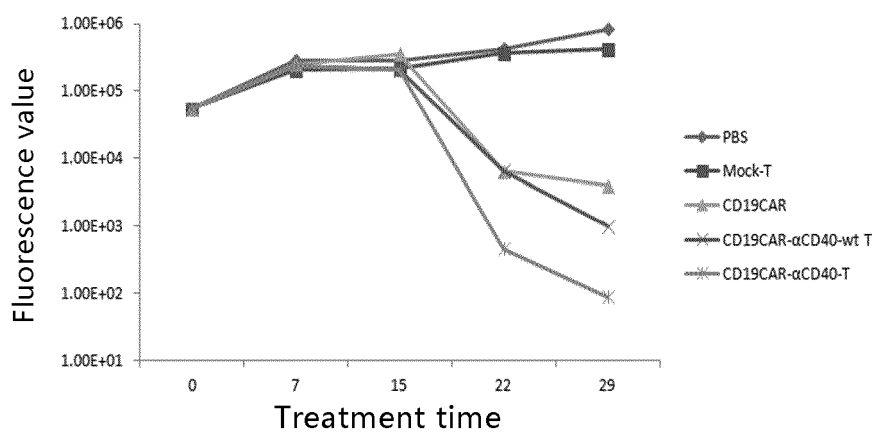


Figure 3

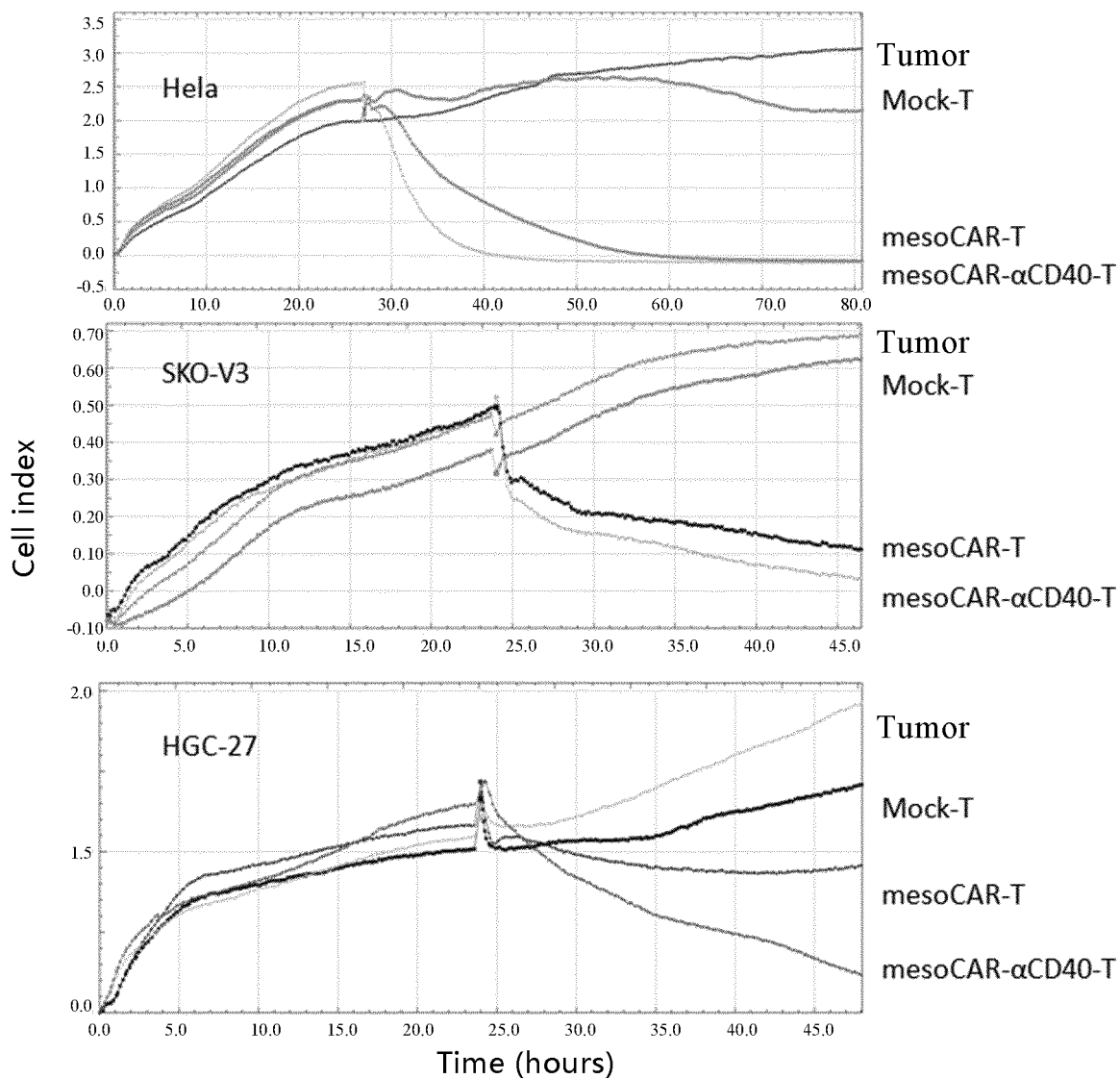


Figure 4
Cytokine secretion

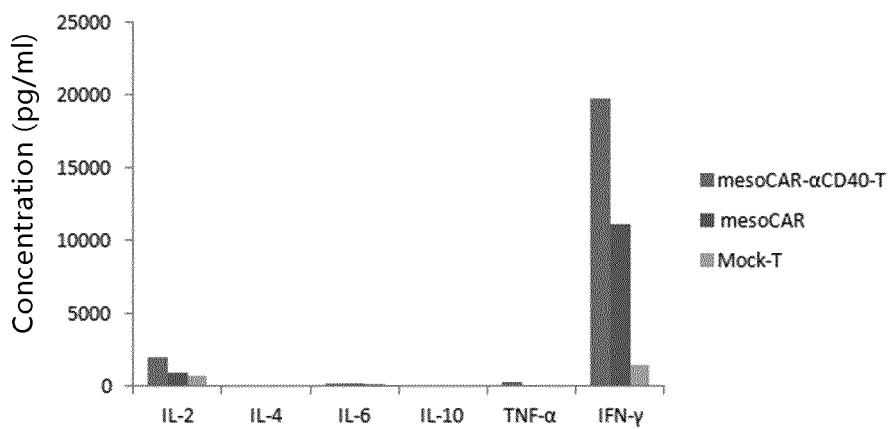


Figure 5

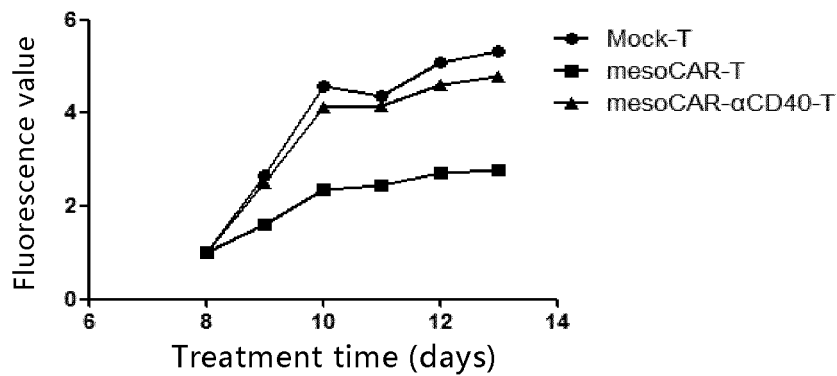


Figure 6

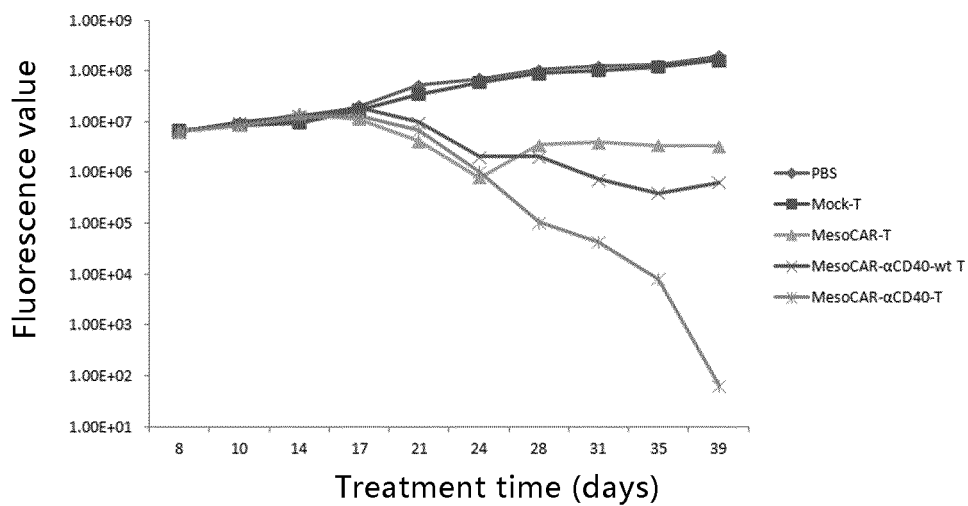


Figure 7

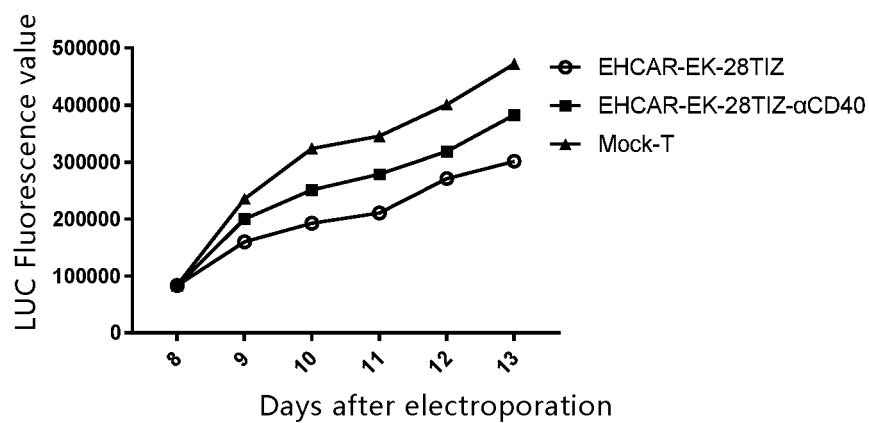


Figure 8

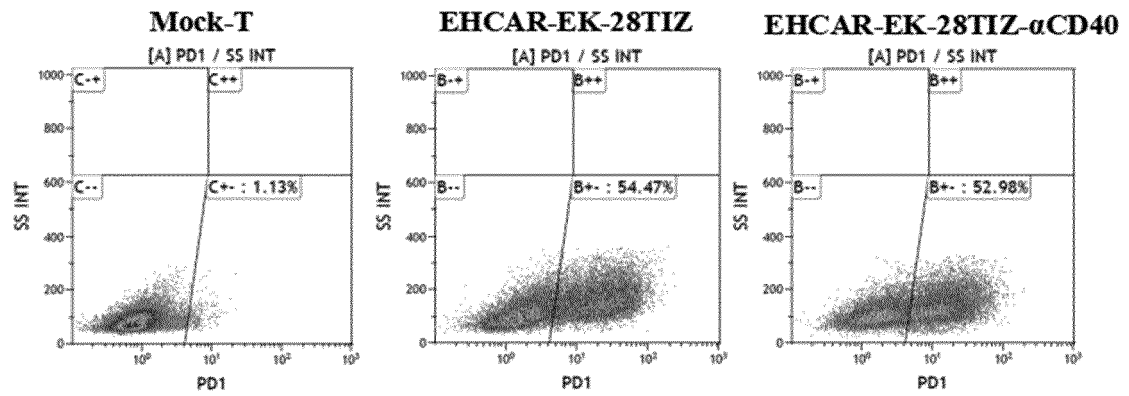


Figure 9A

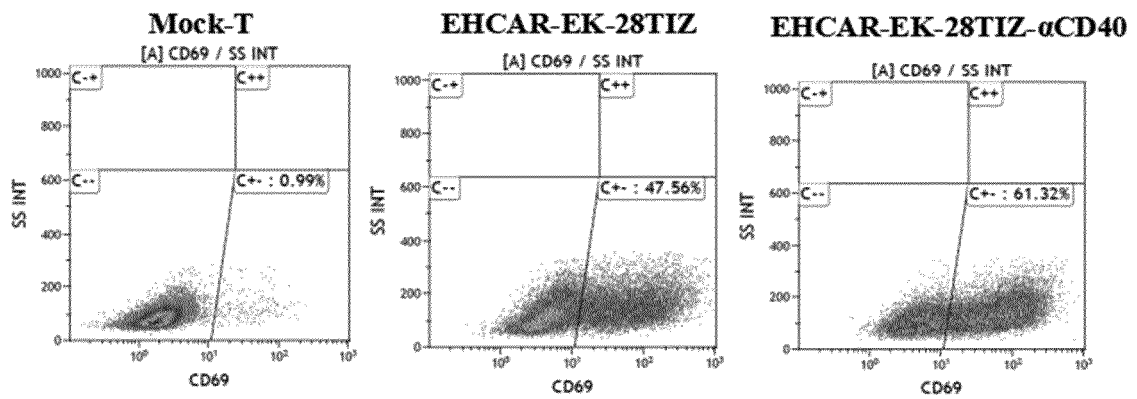


Figure 9B

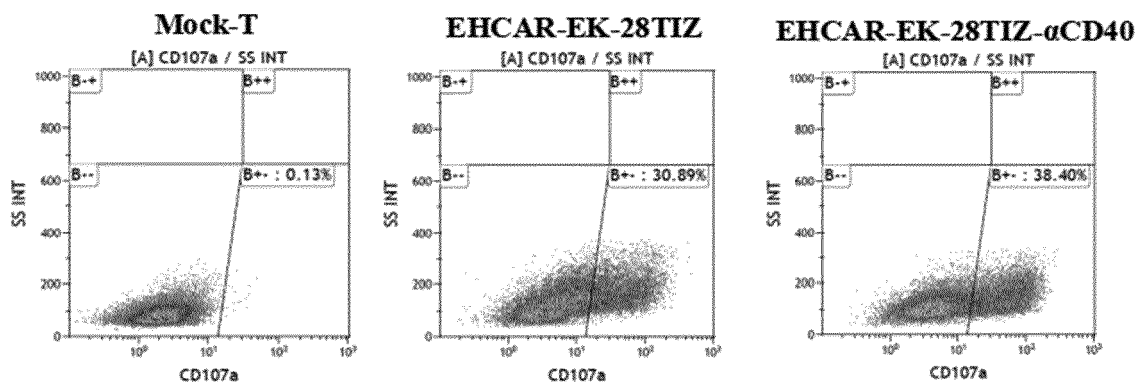
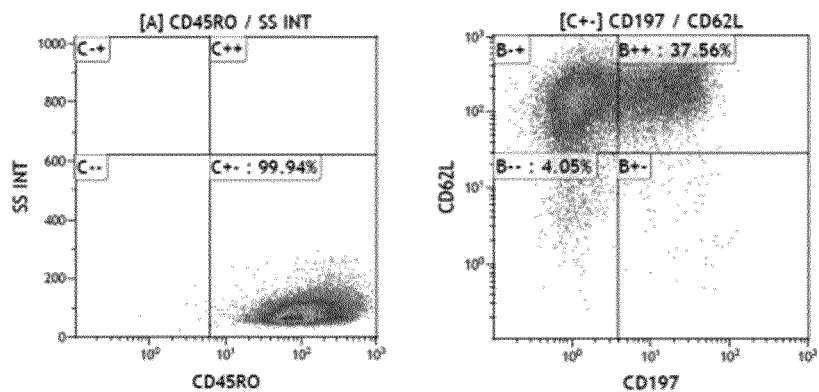
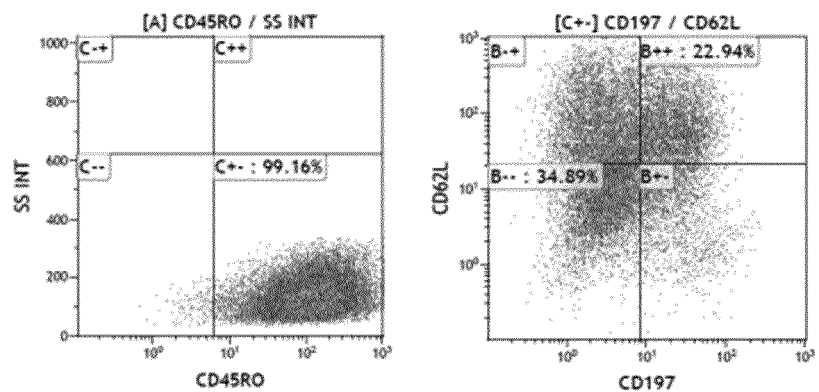


Figure 9C

Mock-T



EHCAR-EK-28TIZ



EHCAR-EK-28TIZ- α CD40

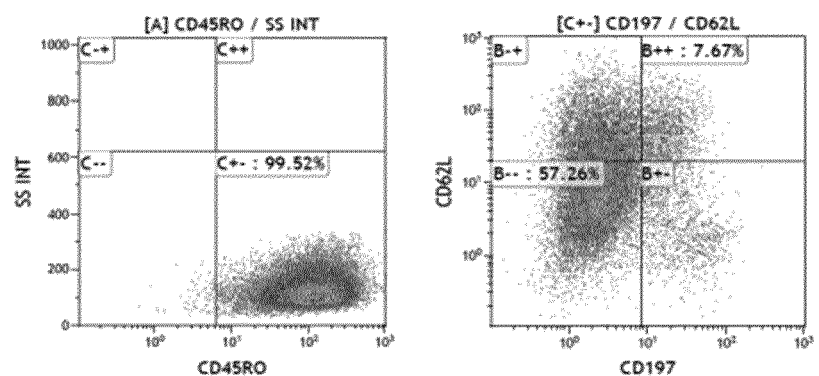


Figure 9D

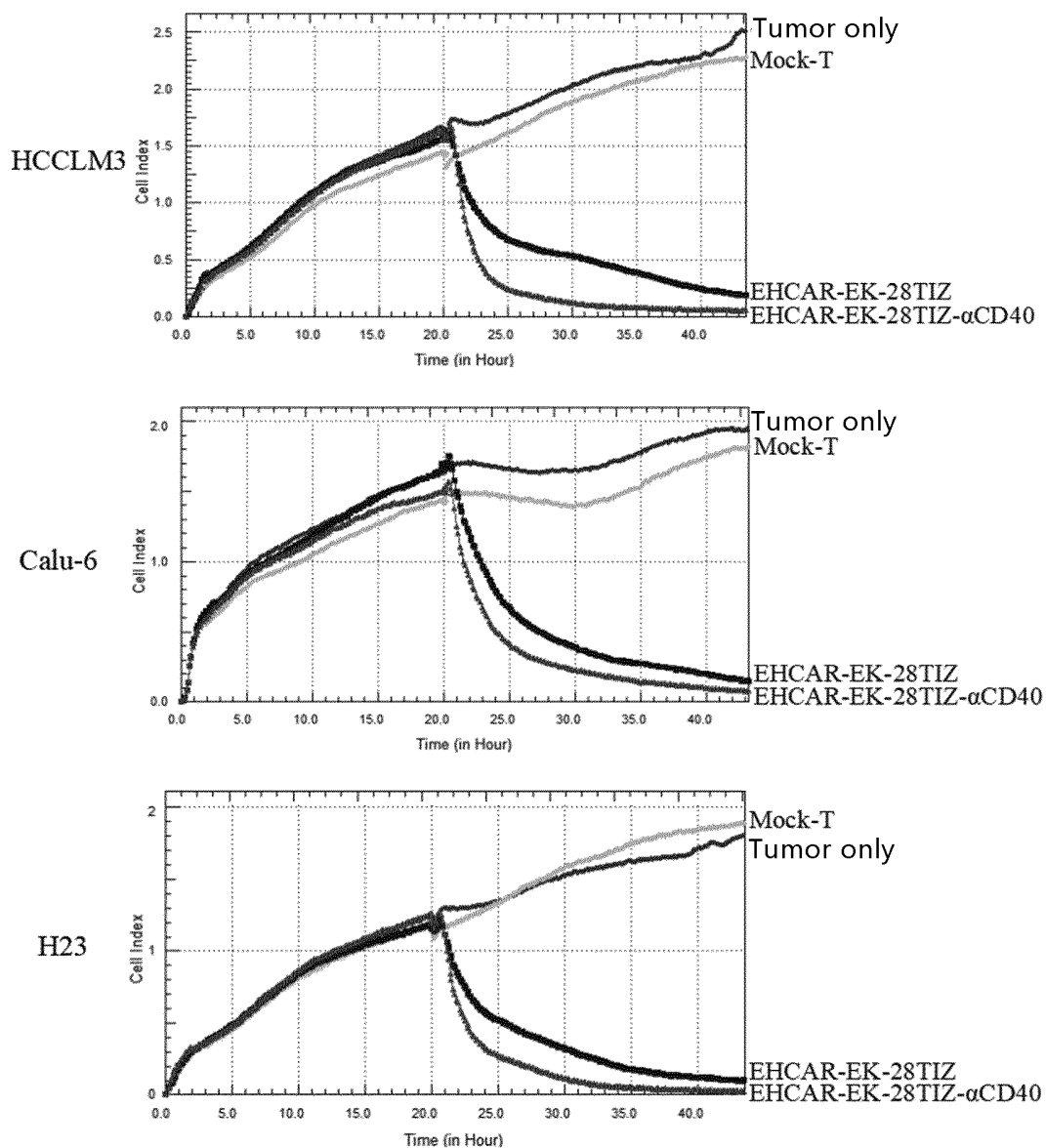


Figure 10

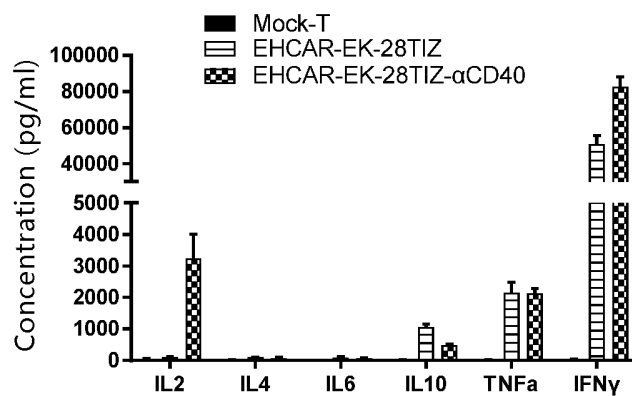


Figure 11

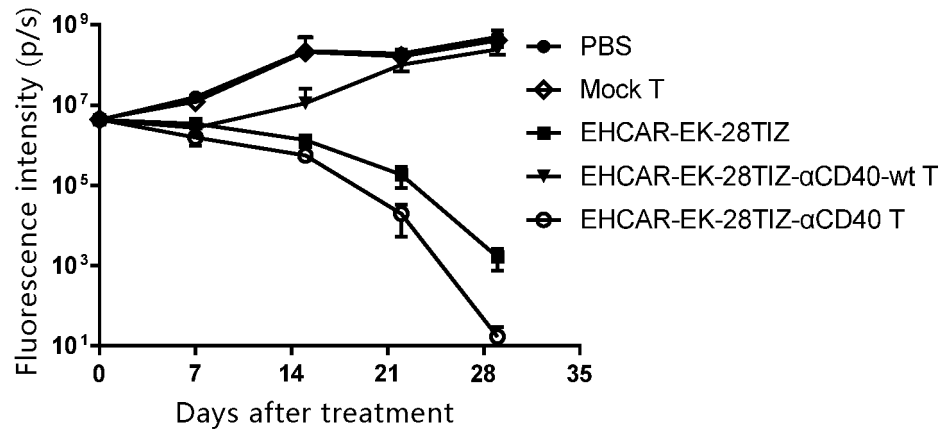


Figure 12

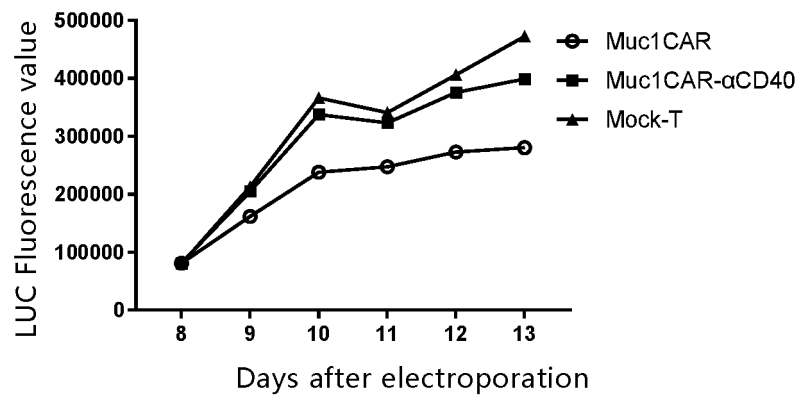


Figure 13

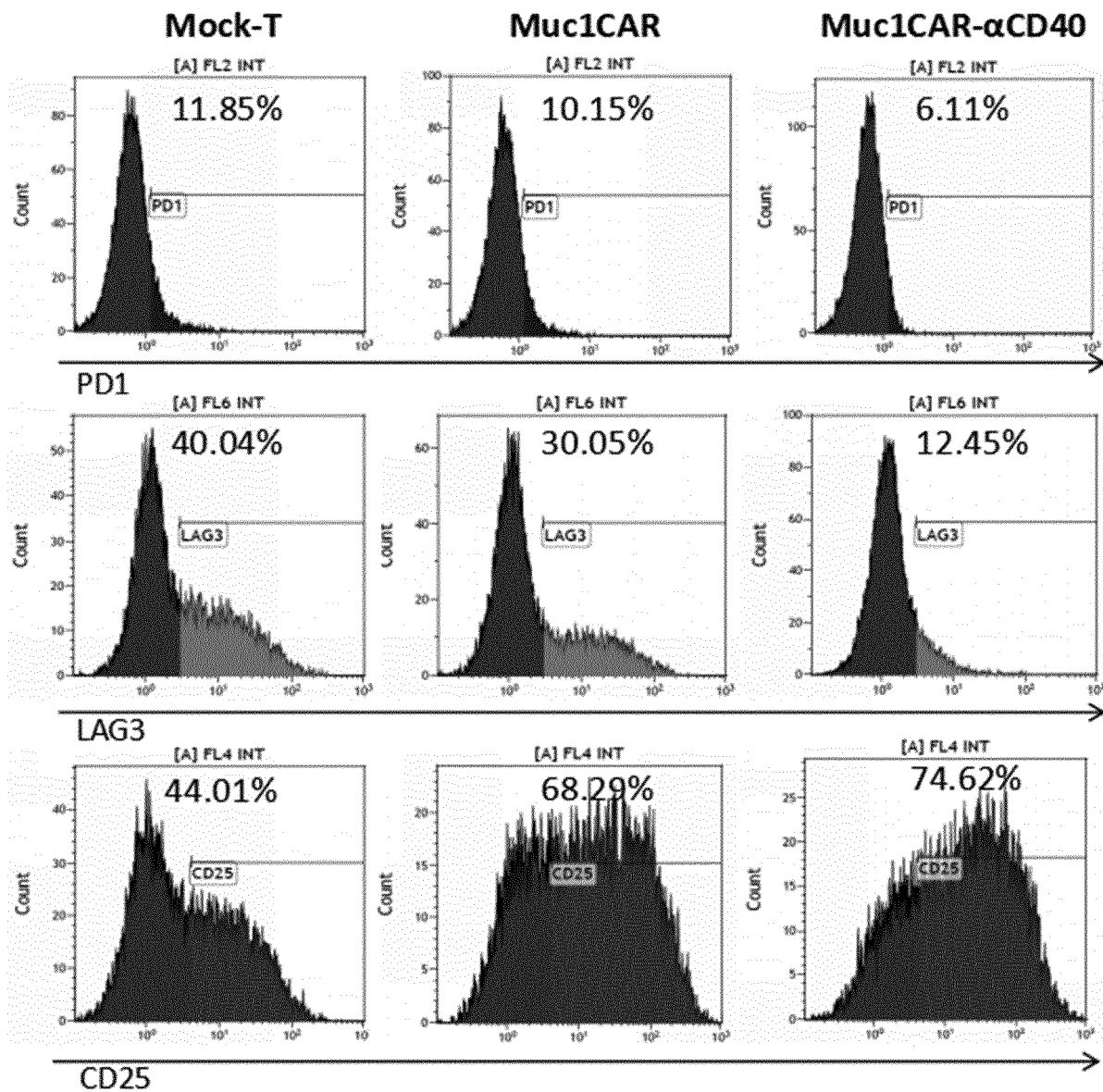


Figure 14A

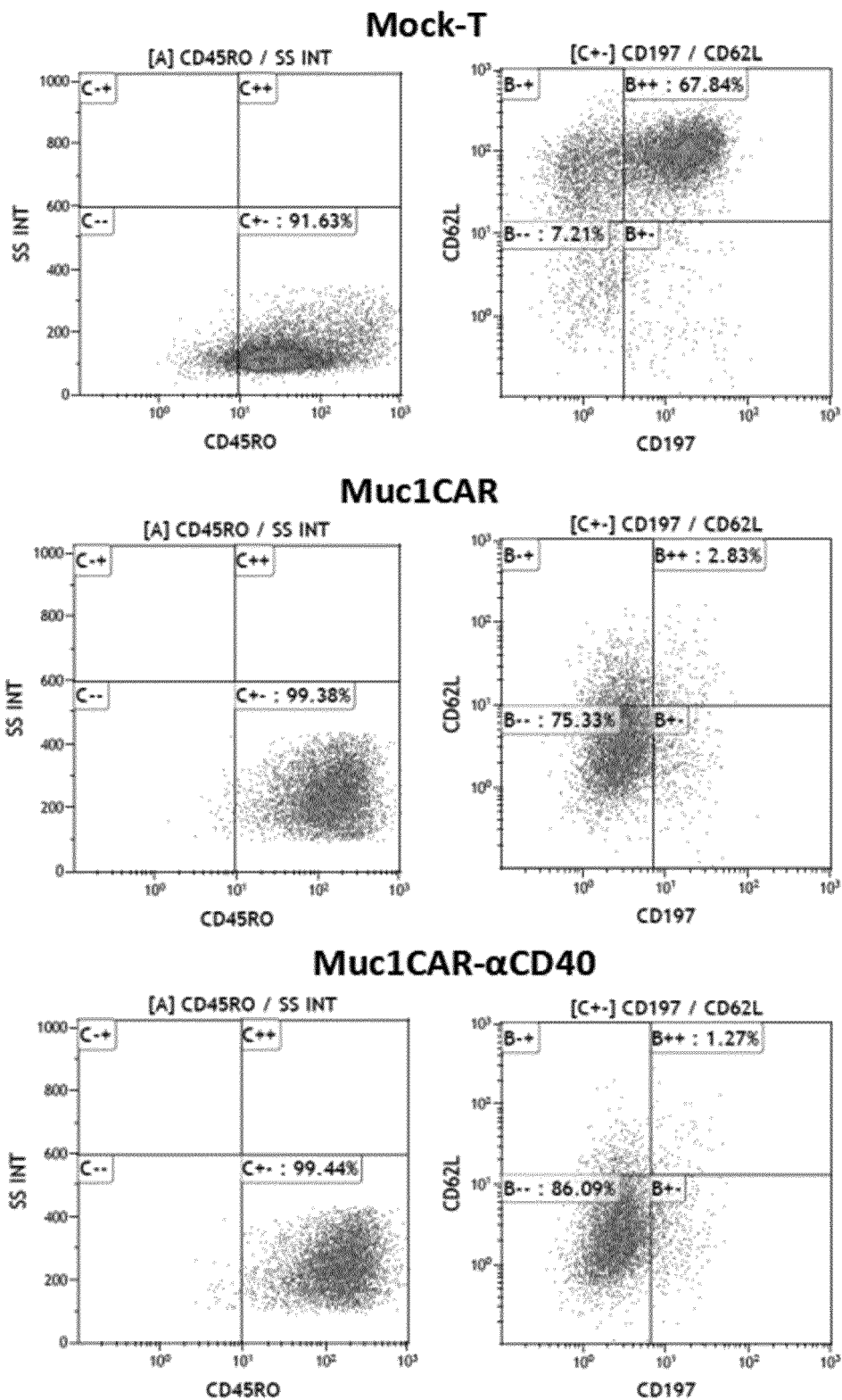


Figure 14B

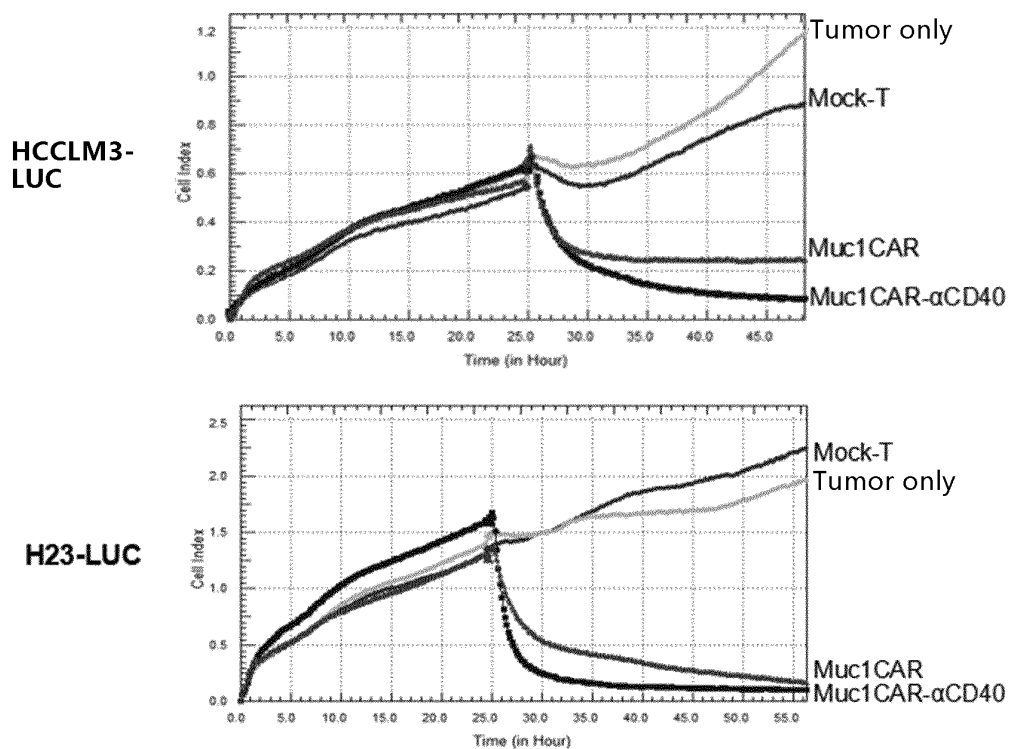


Figure 15

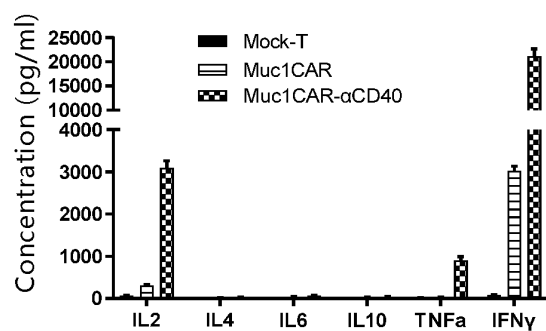


Figure 16

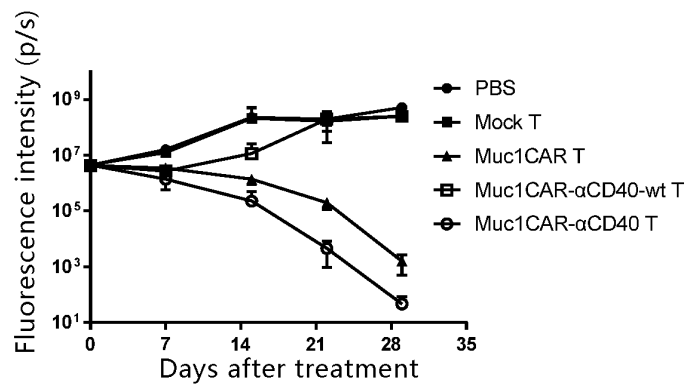


Figure 17

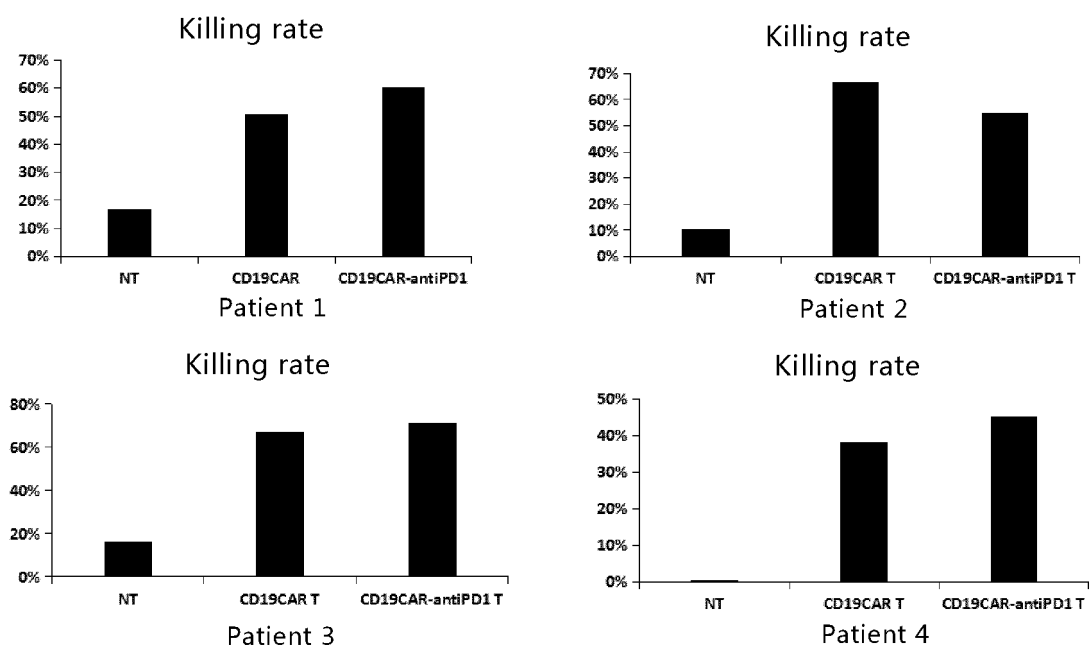


Figure 18

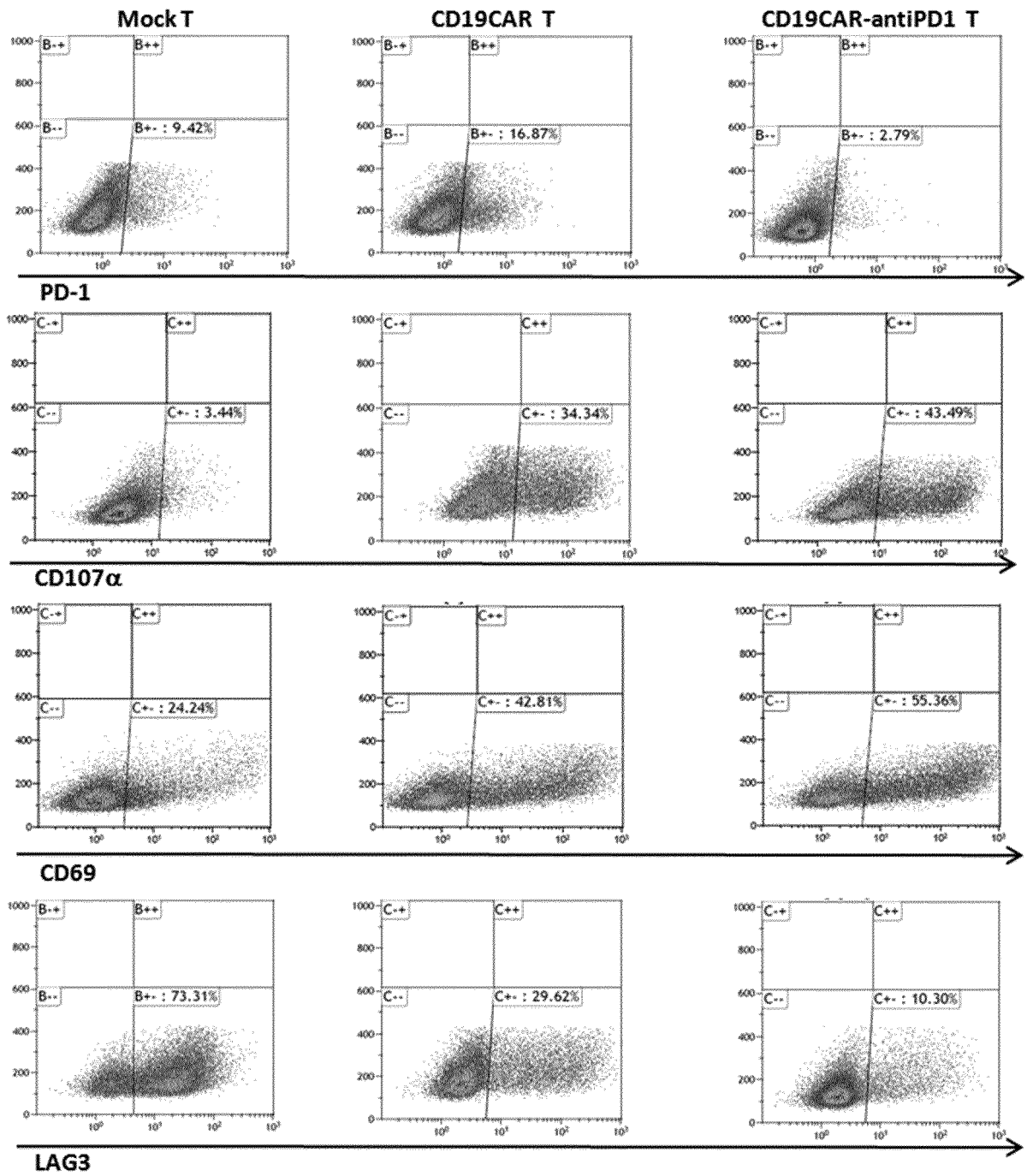


Figure 19

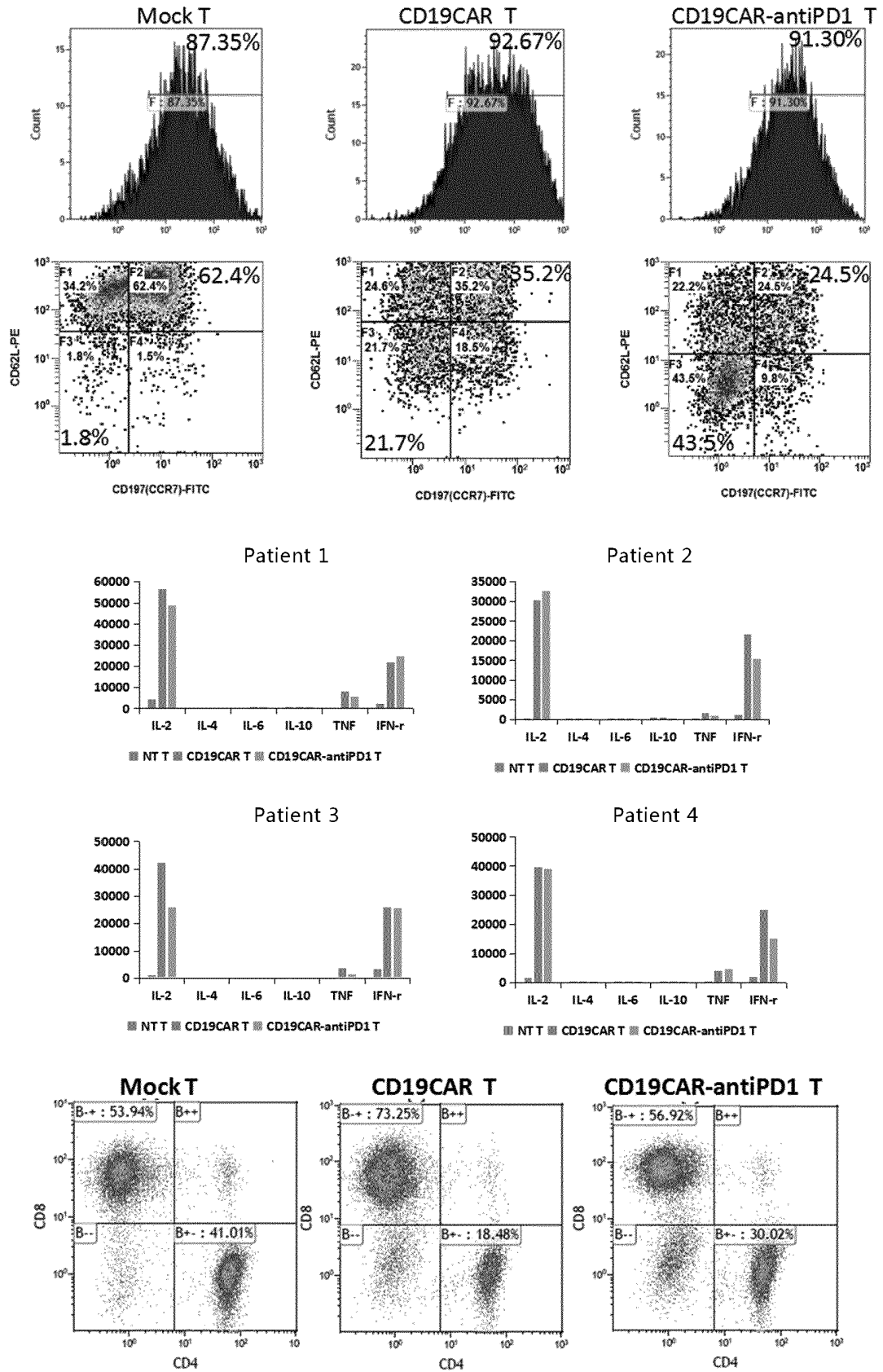


Figure 19 (Continued)

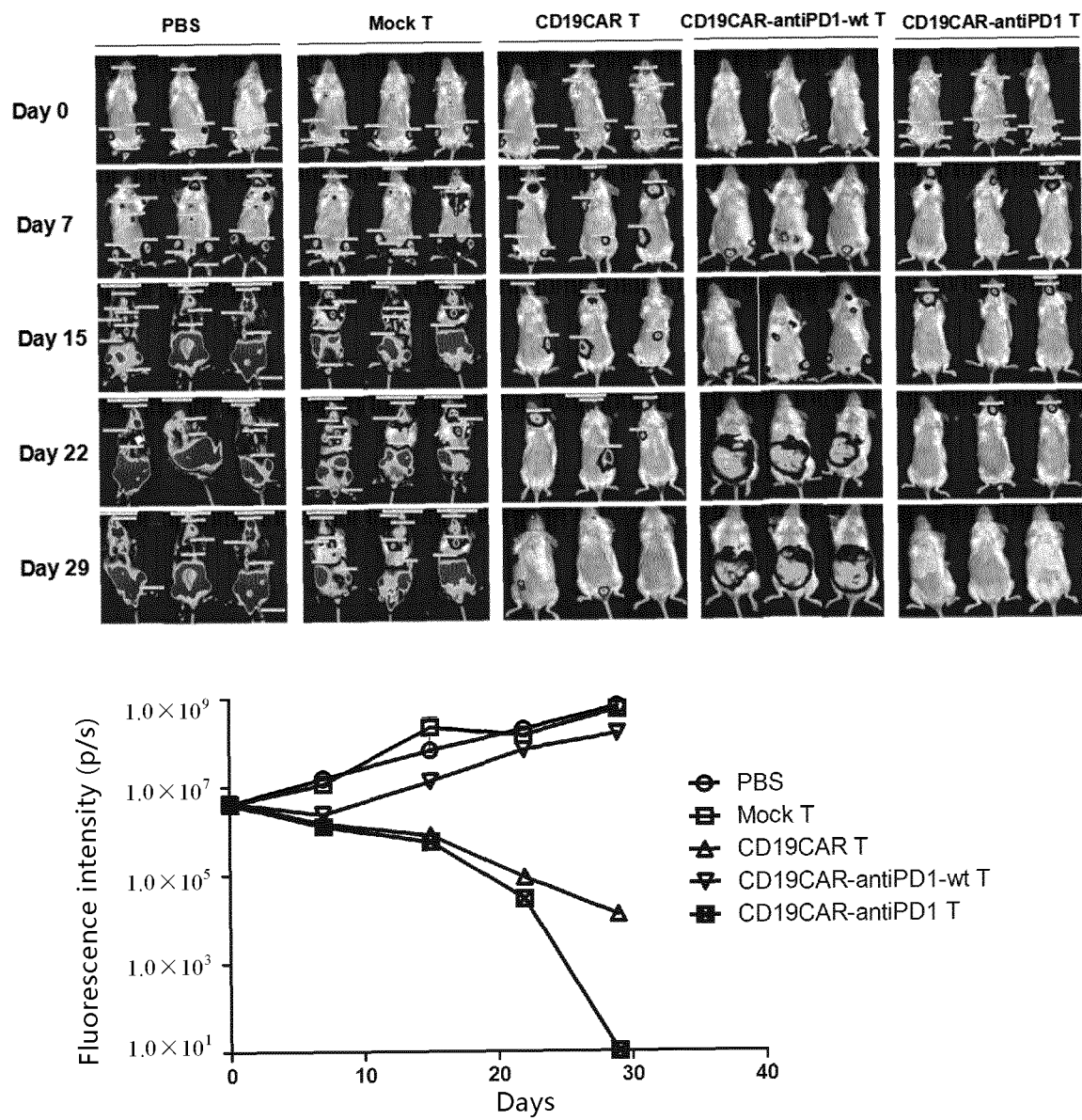


Figure 20

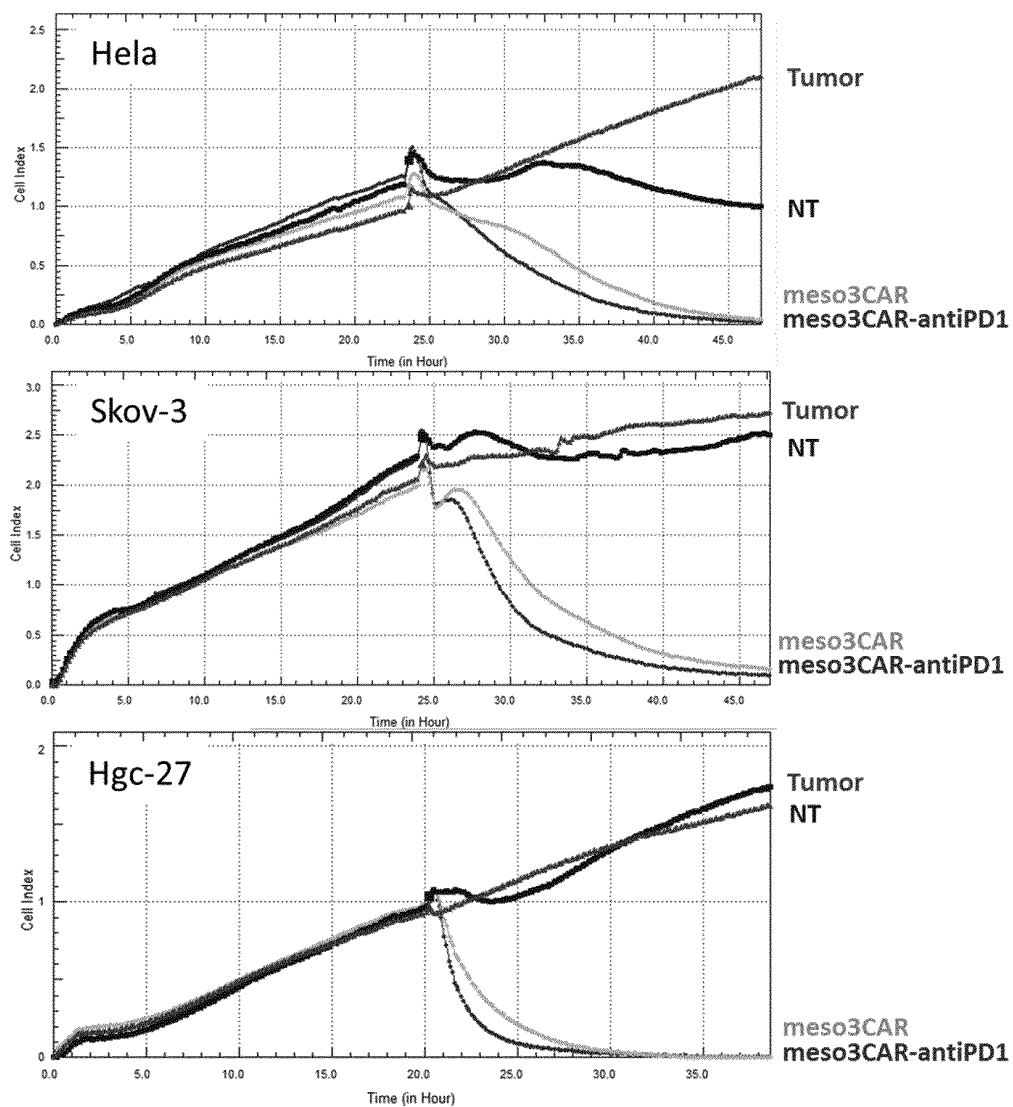


Figure 21

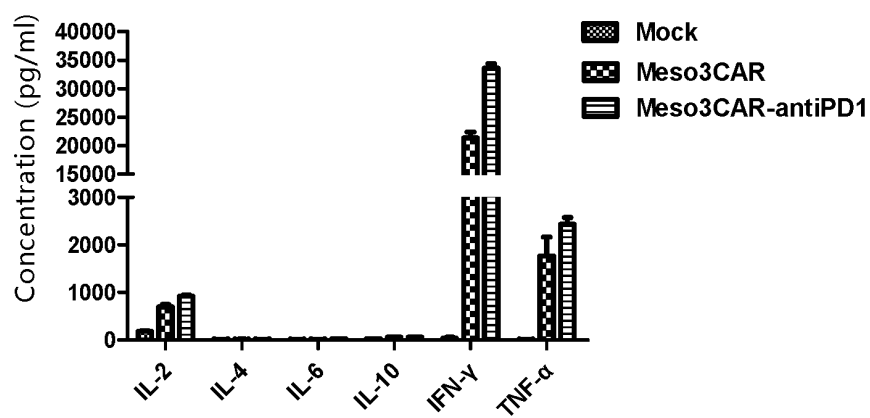


Figure 22

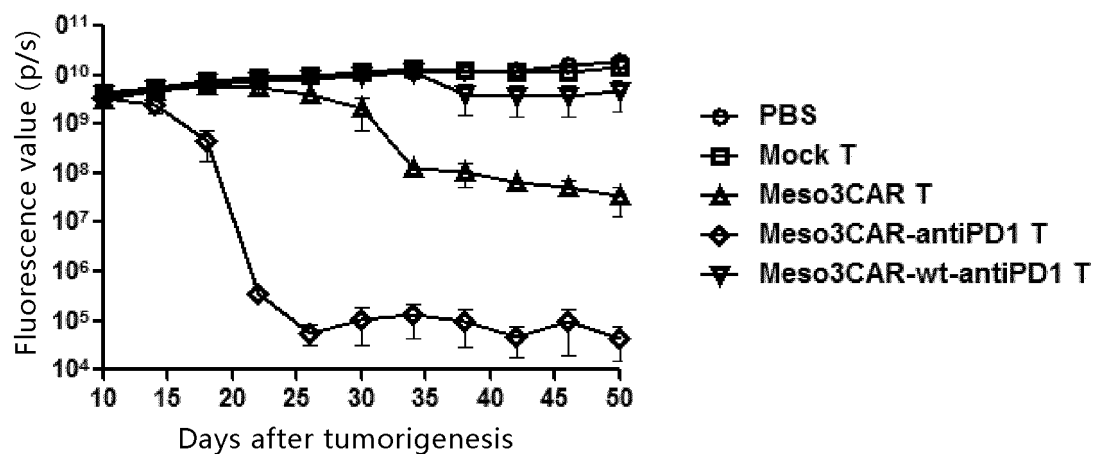


Figure 23

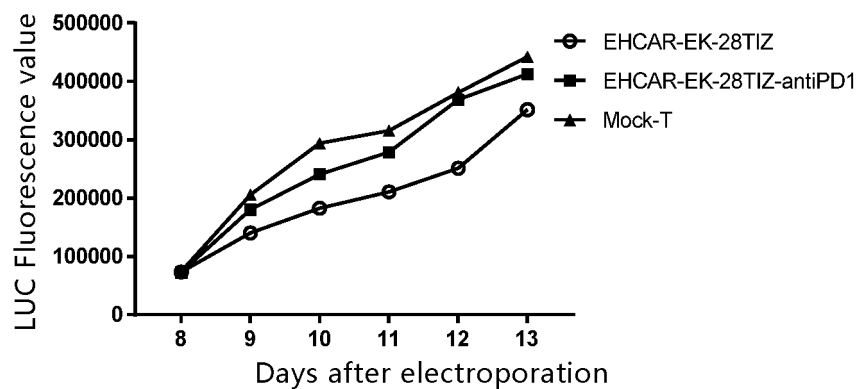


Figure 24

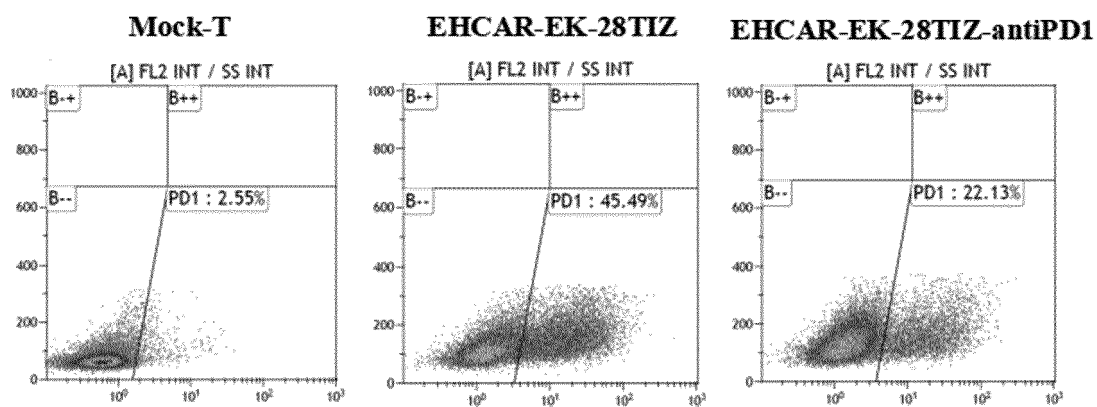


Figure 25A

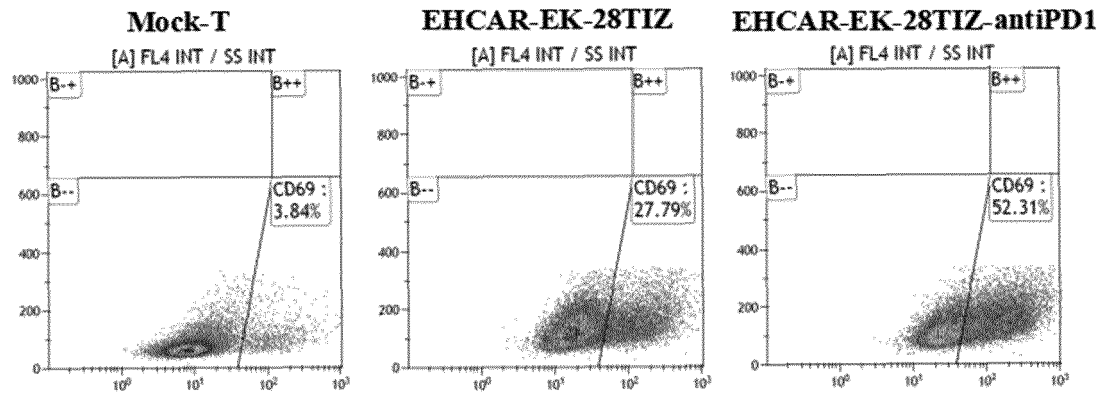


Figure 25B

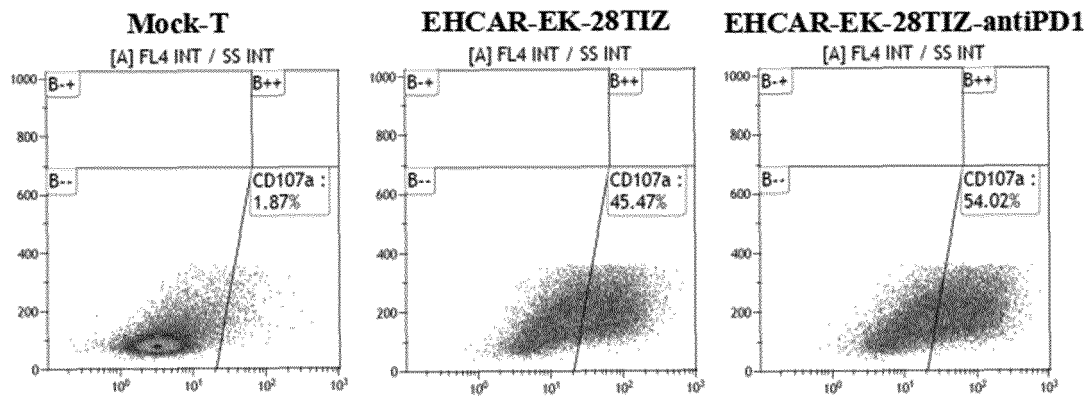
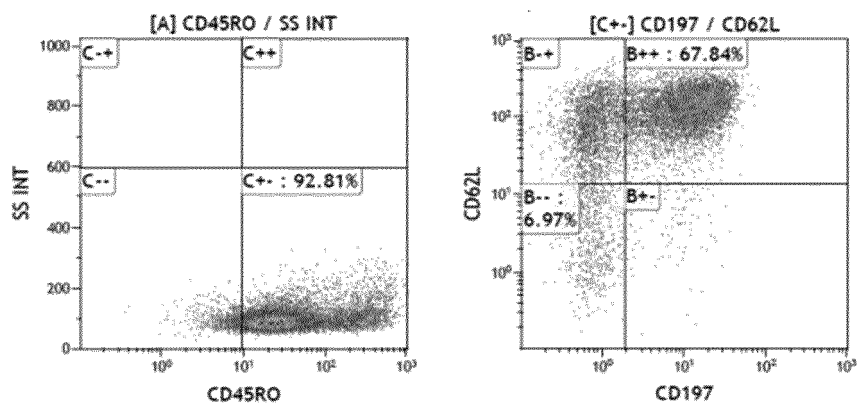
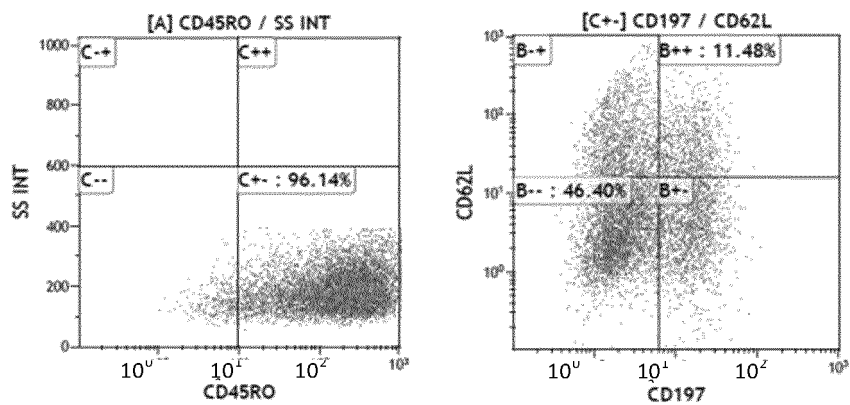


Figure 25C

Mock-T



EHCAR-EK-28TIZ



EHCAR-EK-28TIZ-antiPD1

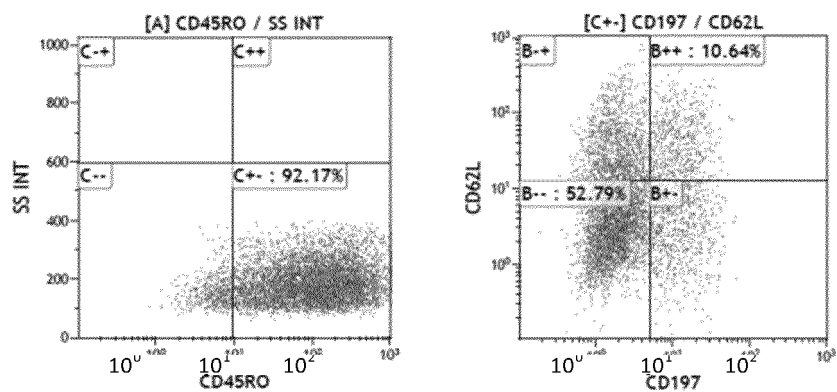


Figure 25D

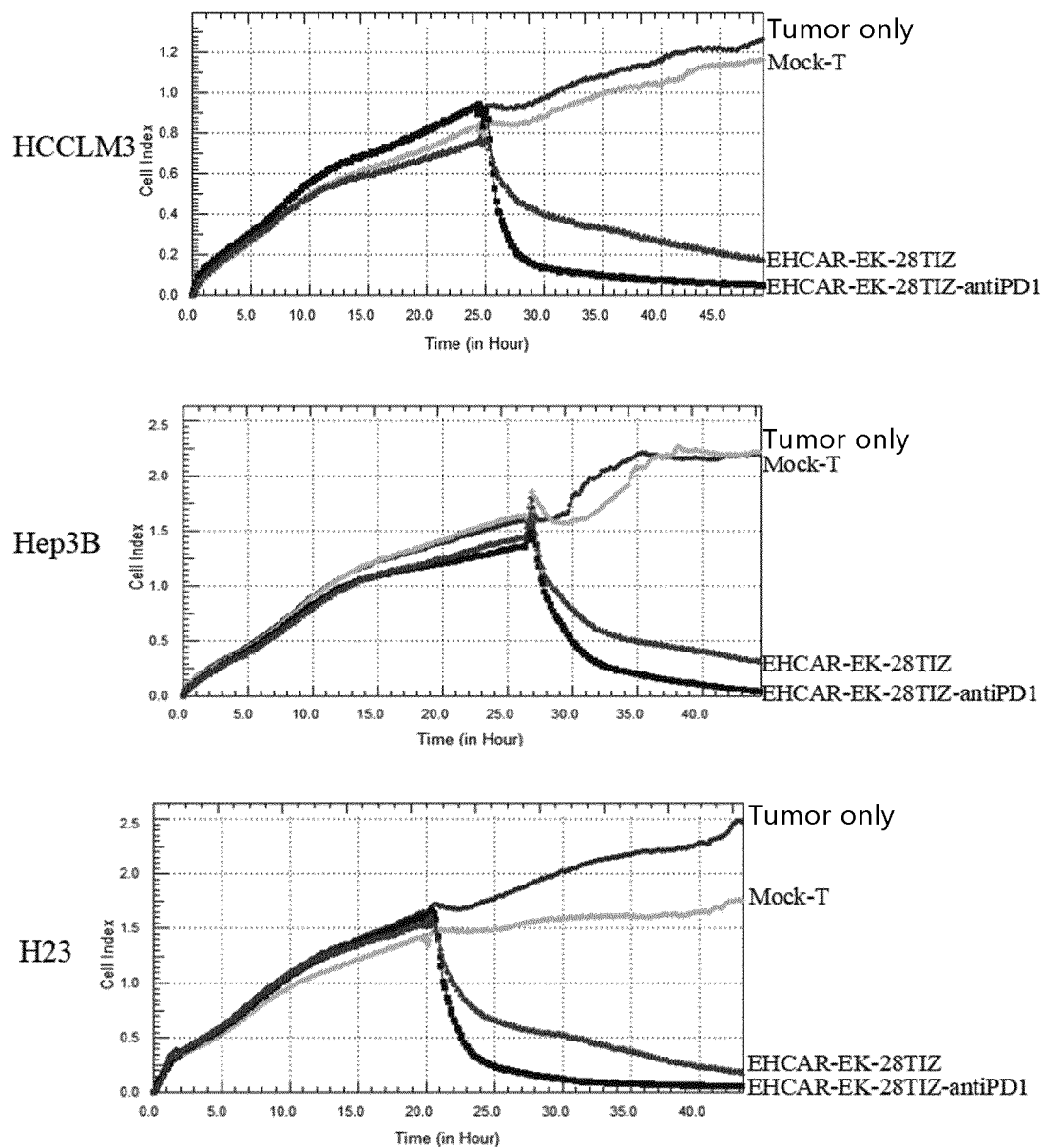


Figure 26

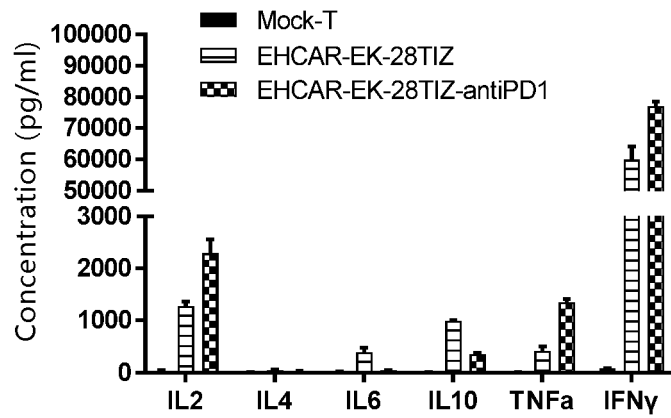


Figure 27

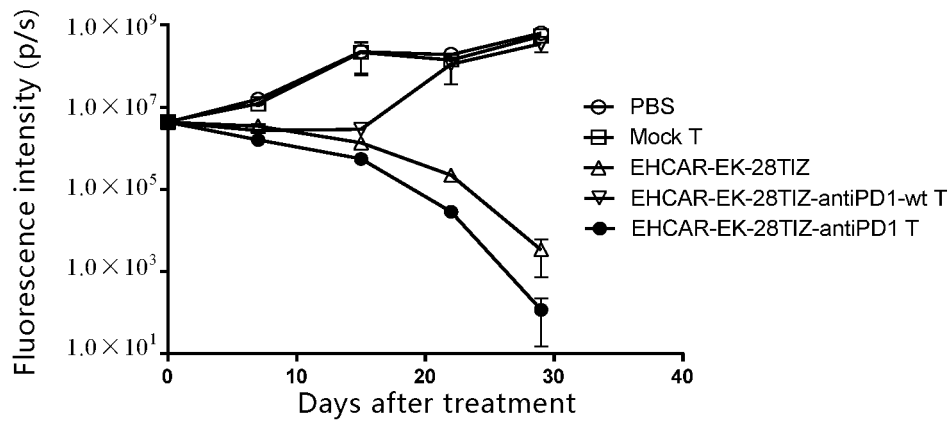


Figure 28

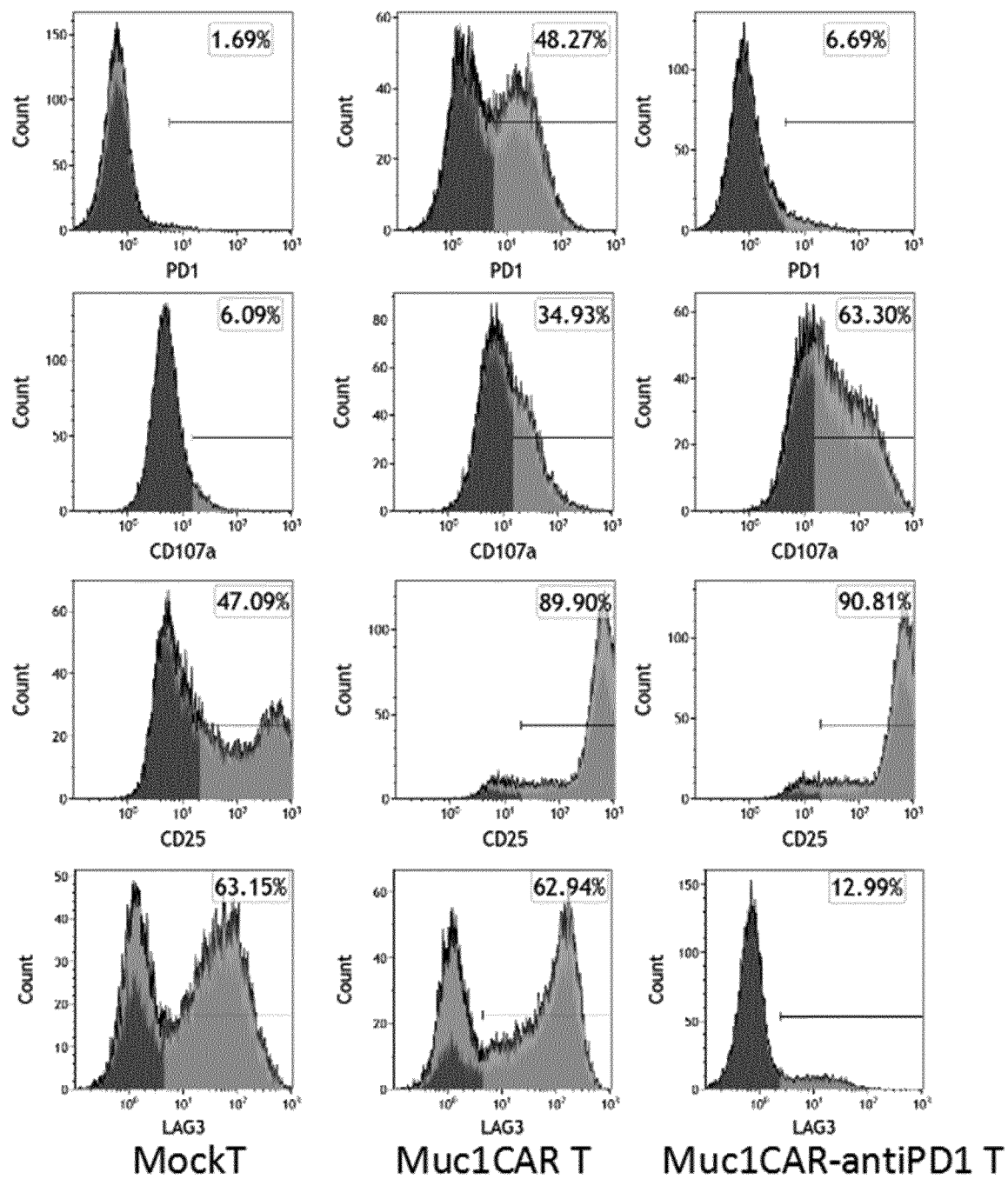


Figure 29A

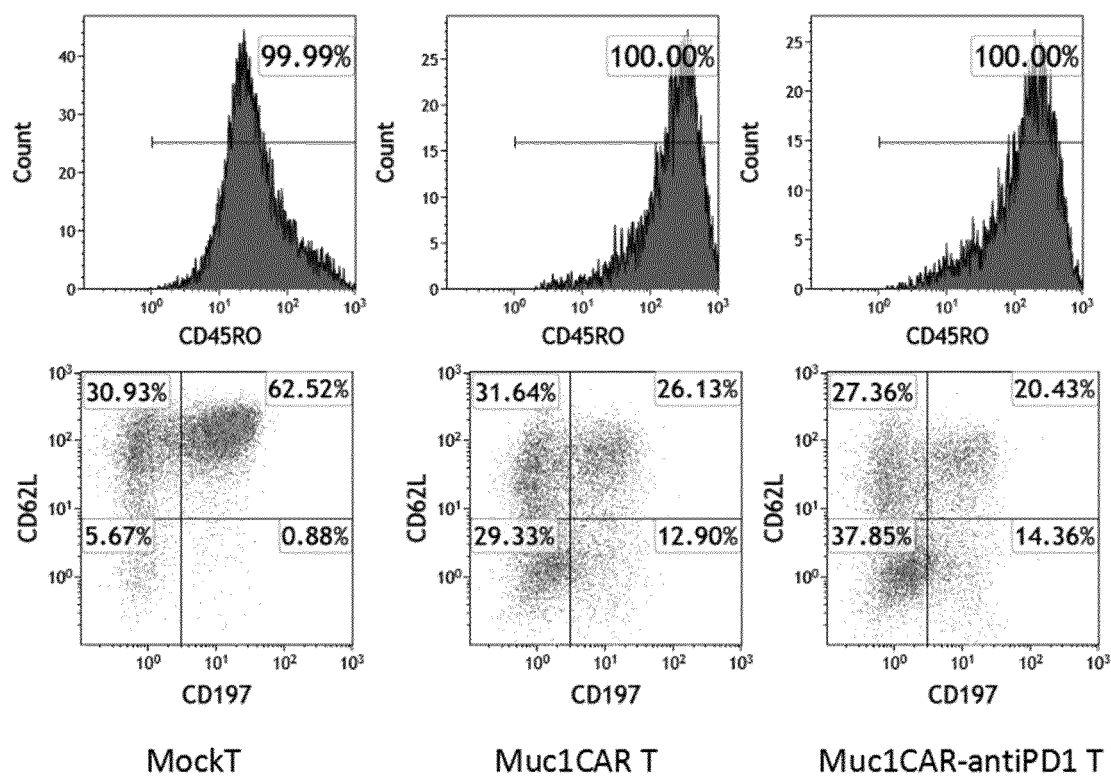


Figure 29B

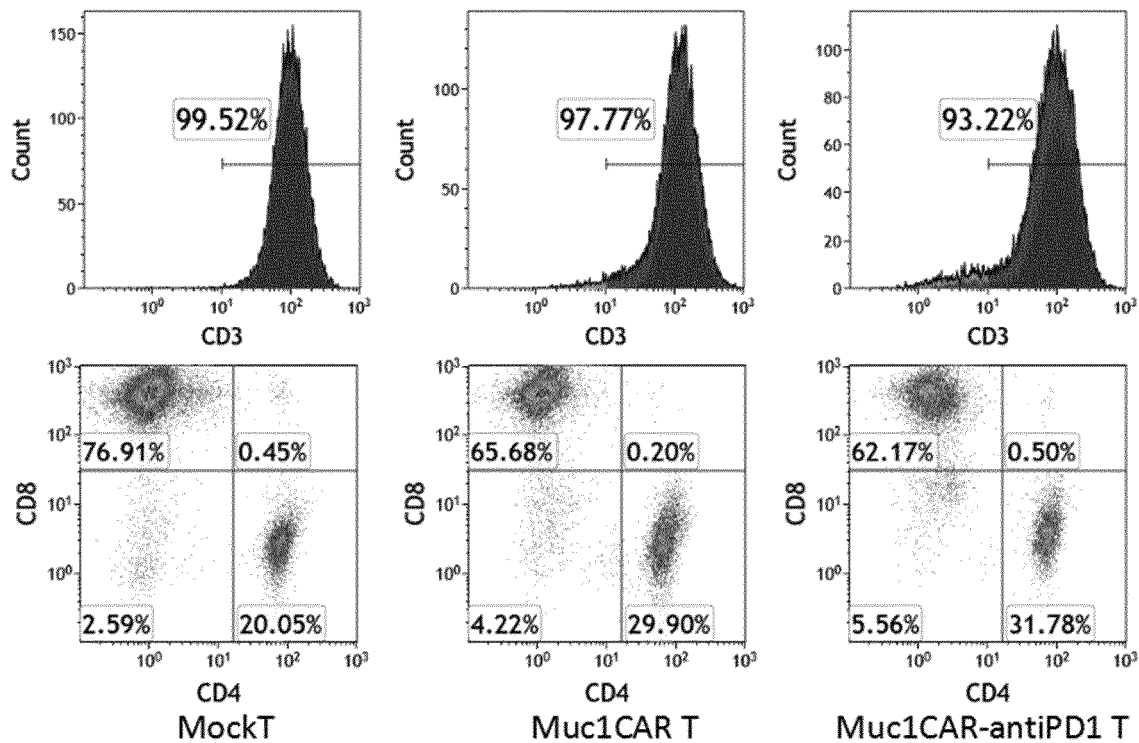


Figure 29C

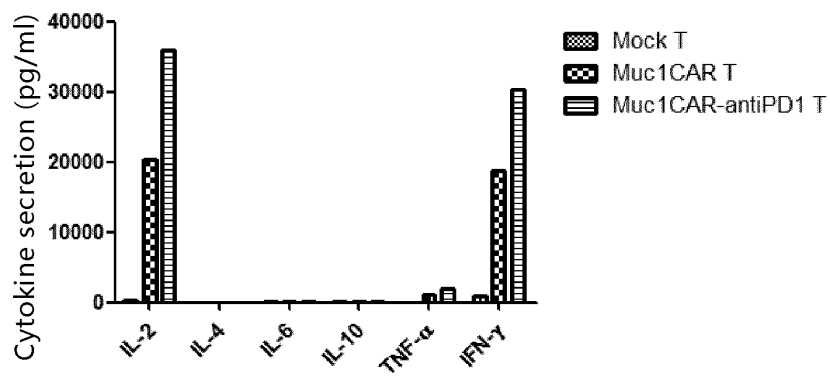


Figure 29D

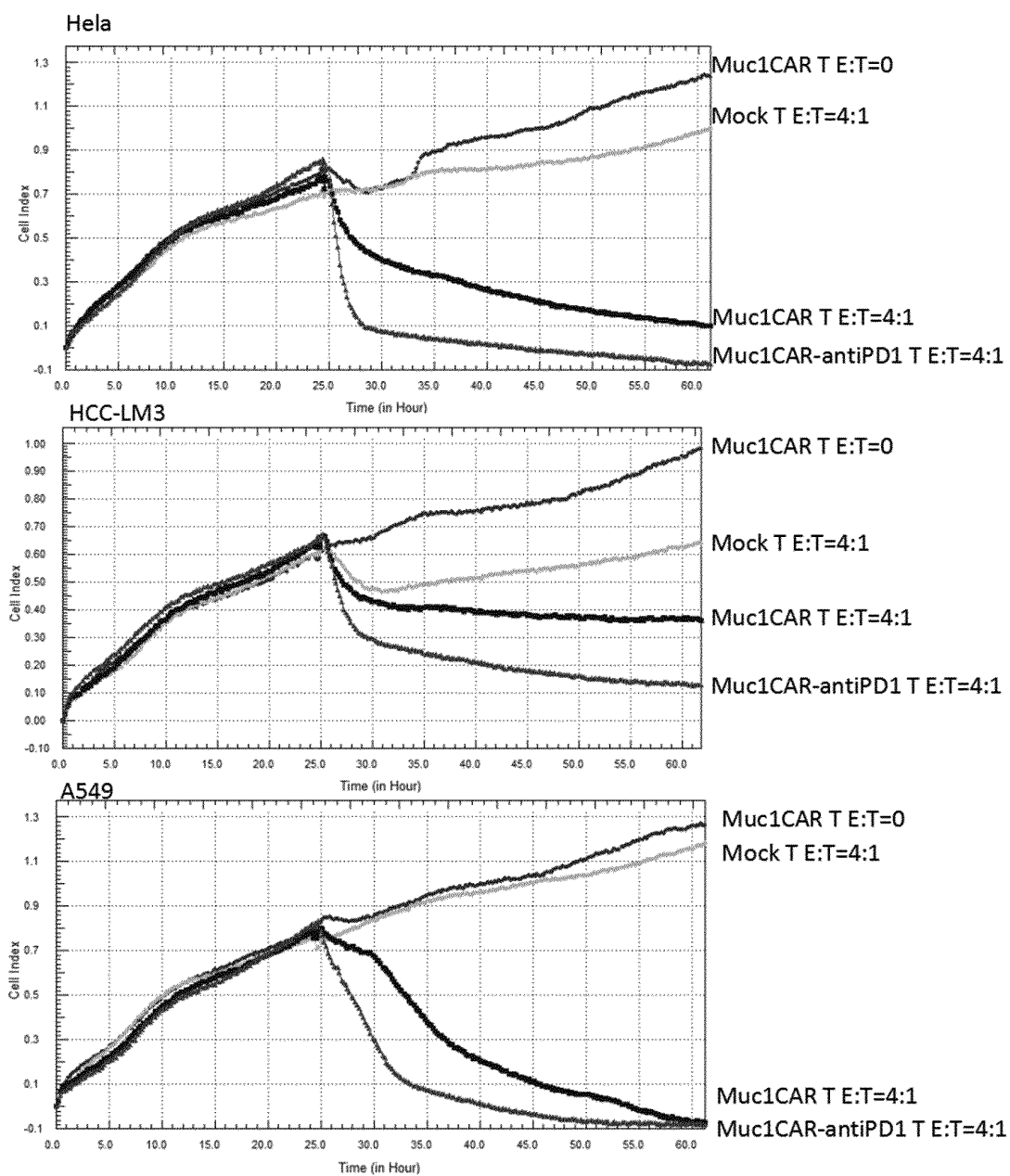


Figure 30

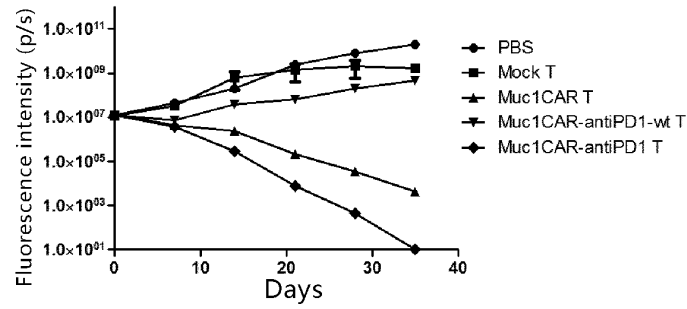


Figure 31

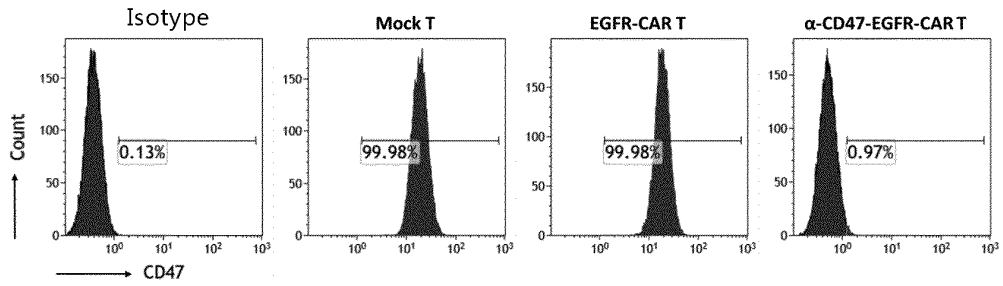


Figure 32

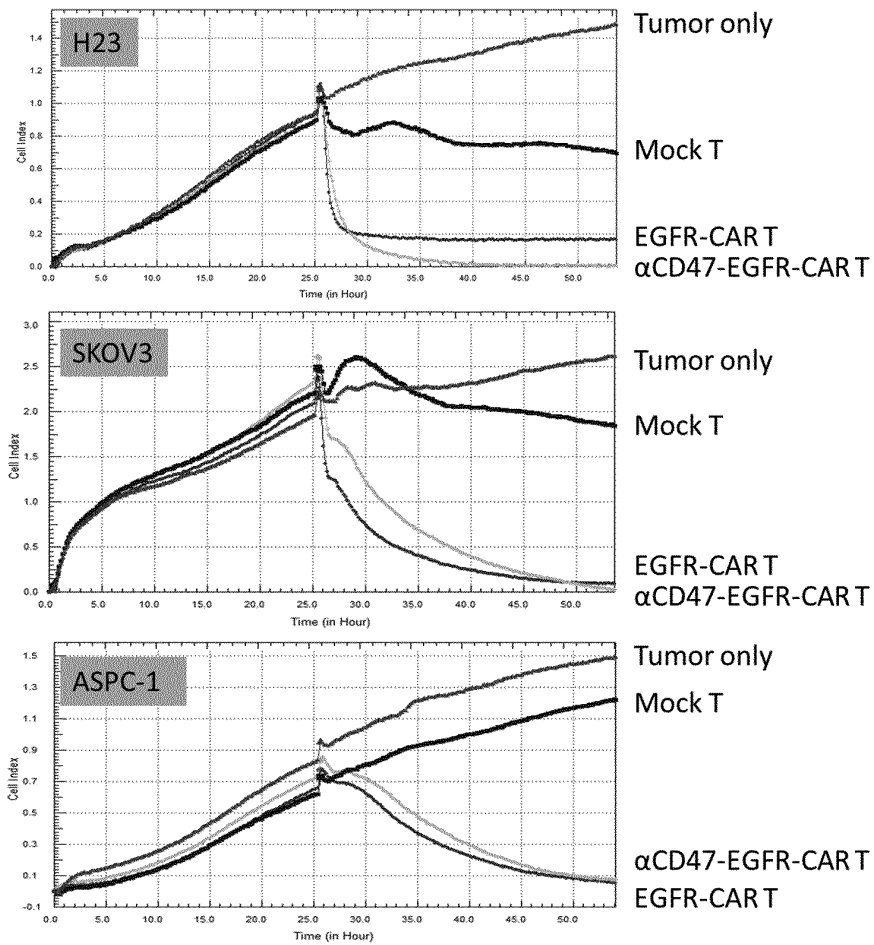


Figure 33

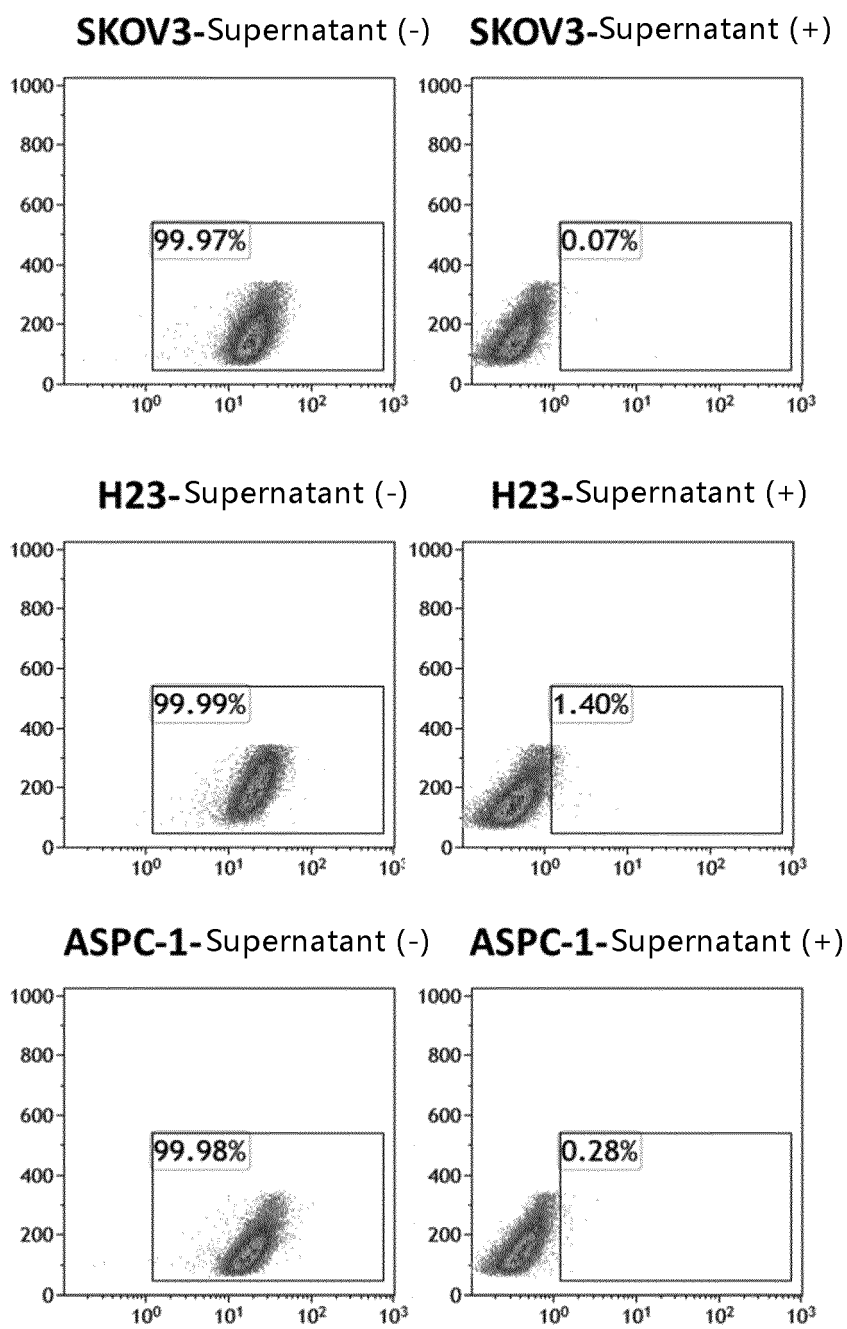


Figure 34

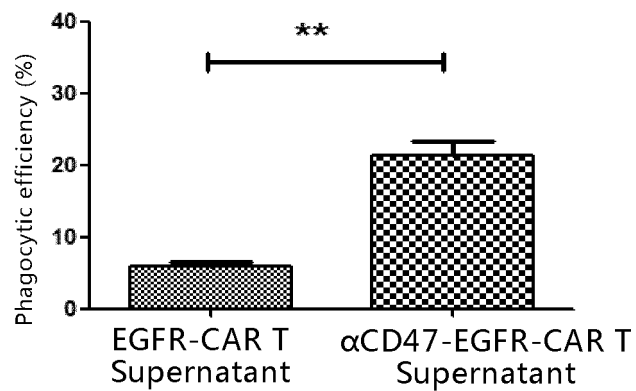


Figure 35

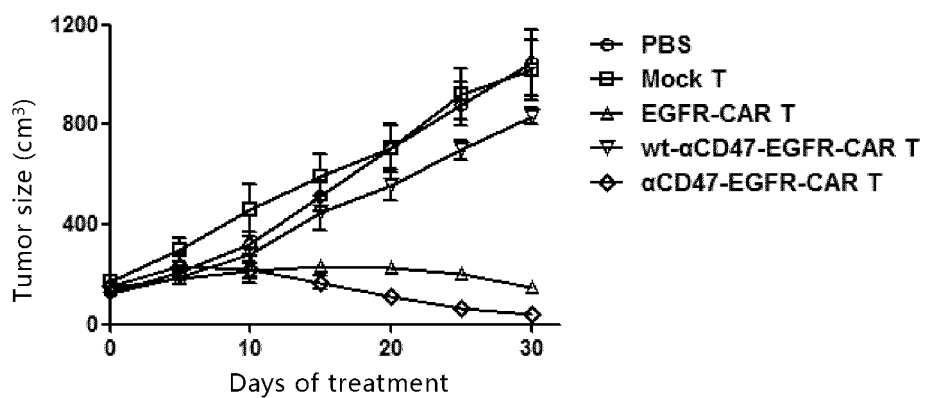


Figure 36

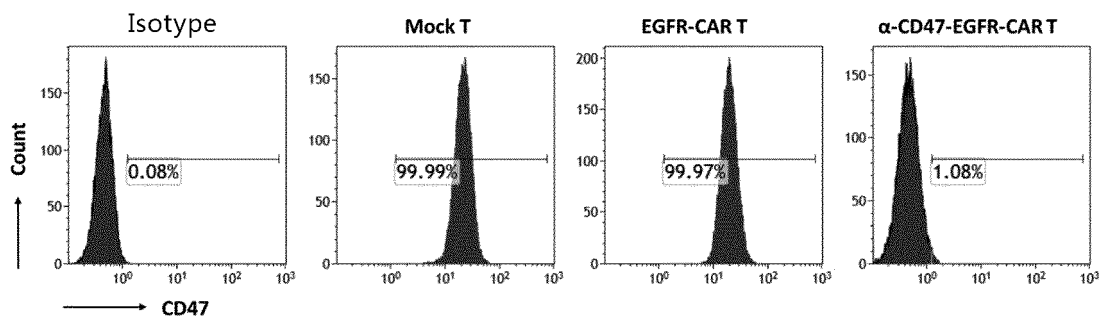


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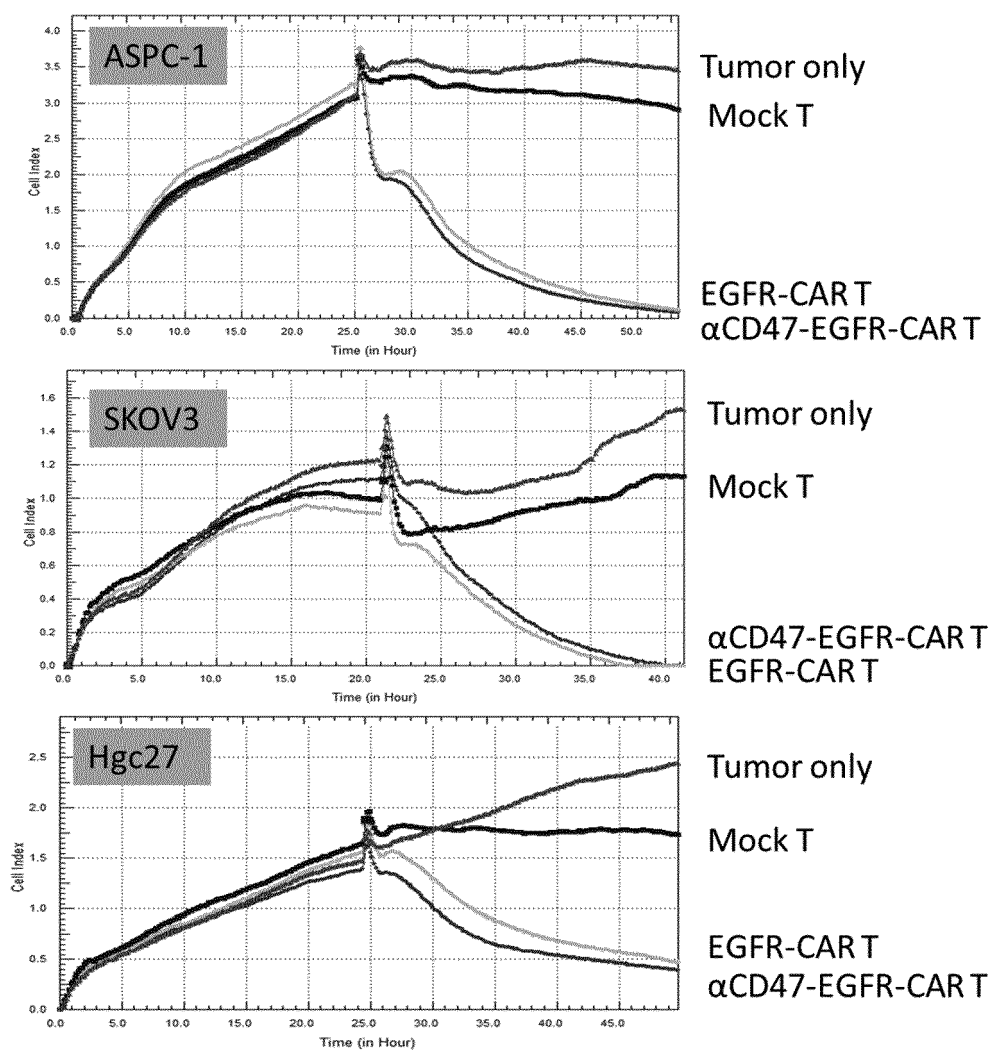


Figure 38

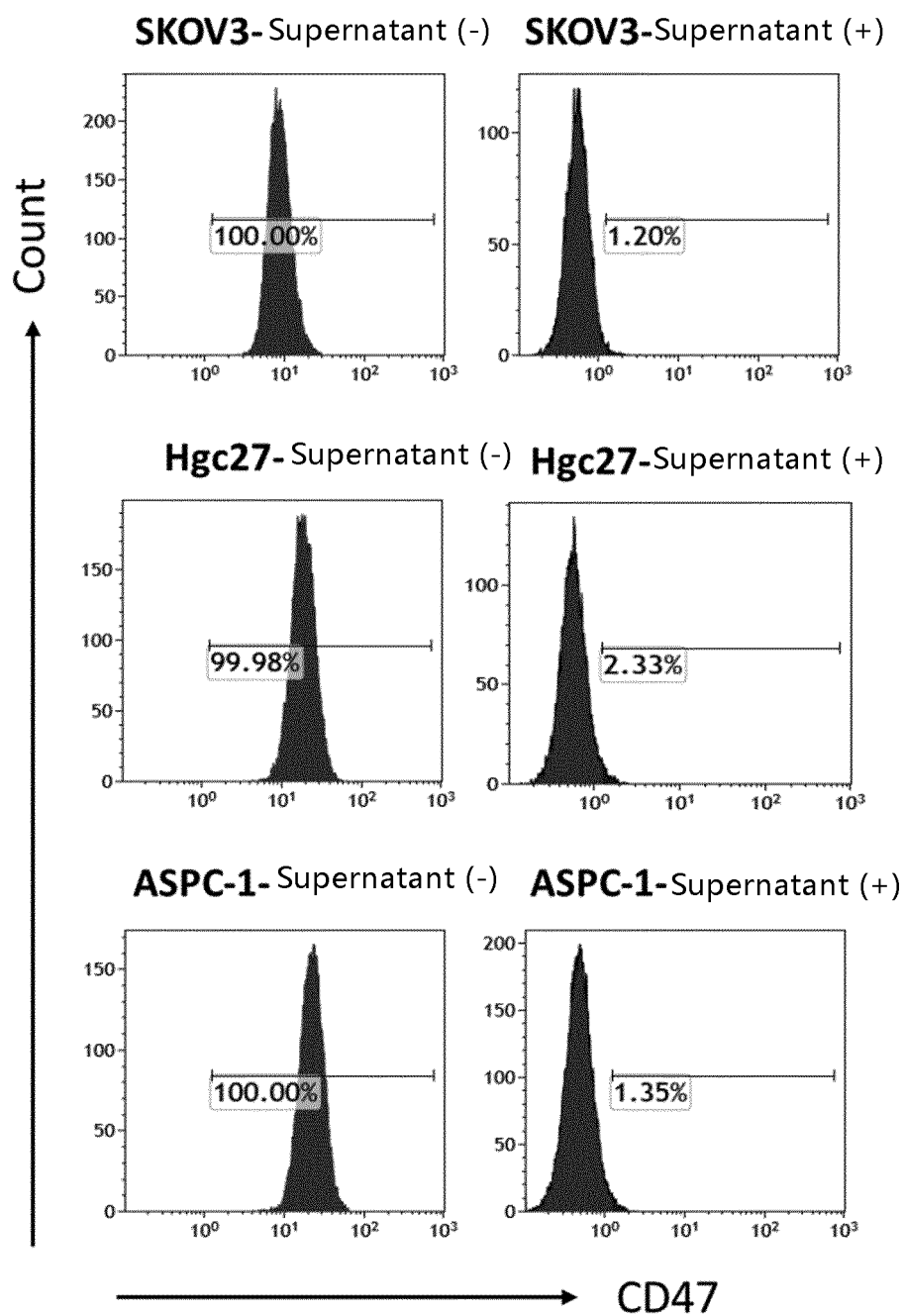


Figure 39

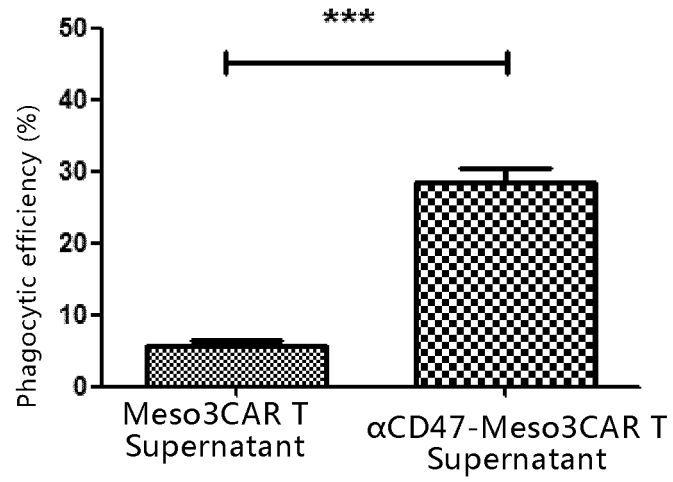


Figure 40

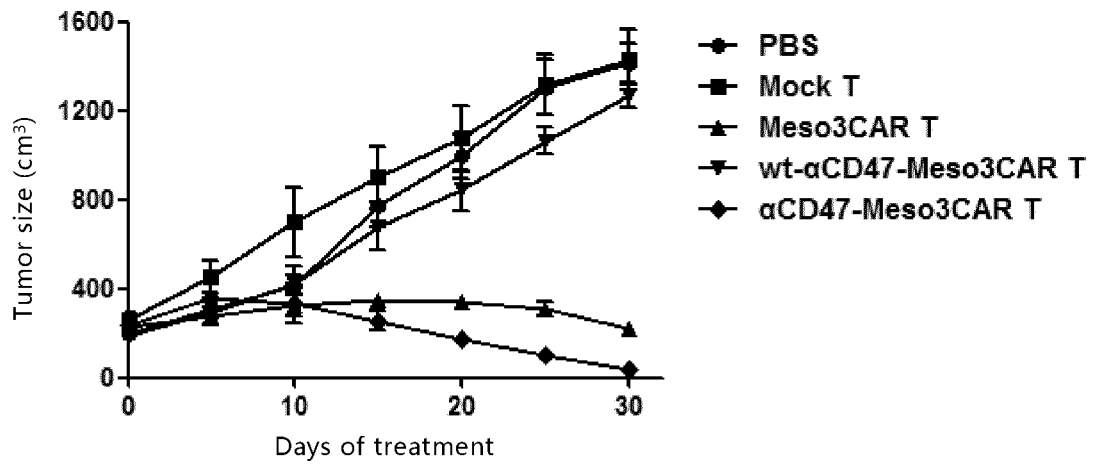


Figure 41

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2018/124692

A. CLASSIFICATION OF SUBJECT MATTER

C12N 5/10(2006.01)i; C12N 15/13(2006.01)i; C12N 15/62(2006.01)i; C12N 15/63(2006.01)i; C07K 19/00(2006.01)i;
C07K 16/28(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)n; A61P 35/02(2006.01)n

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N; C07K; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS; CNTXT; CNKI; USTXT; EPTXT; WOTXT; NCBI; EBI; VEN; ISI web of knowledge; STN; 中国专利生物序列检索系统; T细胞, PD1抗体, PD-1抗体, 信号肽, National Bio-Sequence Database of Chinese Patent: T细胞, PD1抗体, PD-1抗体, 信号肽, search based on sequences 1-25, 突变型Fc, 癌症, 肿瘤, T-cell, PD 1 antibody, PD-1 antibody, signal peptide, SEQ ID NO: 1-25, mutant Fc, cancer, tumor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 107034235 A (YIN, RONG) 11 August 2017 (2017-08-11) description, paragraphs [0007]-[0050]	1-28
Y	CN 1922316 A (KIRIN BREWERY COMPANY, LIMITED) 28 February 2007 (2007-02-28) abstract, claim 1, and sequence listing 139	1-28
Y	CN 106414503 A (MOLECULAR TEMPLATES, INC.) 15 February 2017 (2017-02-15) claim 5	12, 21-24, 26-28
A	CN 105950561 A (JIANGSU JIESHENG BIOSCIENCE CO., LTD.) 21 September 2016 (2016-09-21) entire document	1-28

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

17 March 2019

Date of mailing of the international search report

27 March 2019

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing
100088
China

Authorized officer

Facsimile No. (86-10)62019451

Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2018/124692

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2018/124692

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 107034235 A	11 August 2017	None	
CN 1922316 A	28 February 2007	KR 20060130615 A	19 December 2006
		TW 200540186 A	16 December 2005
		US 2007148163 A1	28 June 2007
		US 9023361 B2	05 May 2015
		HR P20130078 T1	28 February 2013
		WO 2005063981 A1	14 July 2005
		NO 20063197 A	21 September 2006
		ZA 200605874 A	25 June 2008
		JP 2009022289 A	05 February 2009
		US 9023360 B2	05 May 2015
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		AU 2004309275 A1	14 July 2005
		DK 1707627 T3	17 December 2012
		JP 4242388 B2	25 March 2009
		IL 176508 D0	05 October 2006
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		RU 2377254 C2	27 December 2009
		CN 1922316 B	23 March 2011
		US 9598494 B2	21 March 2017
		SI EP1707627 T1	28 February 2013
		KR 20120116018 A	19 October 2012
		AU 2004309275 B2	23 December 2010
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		EP 1707627 A1	04 October 2006
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		HK 1102134 A1	03 June 2011
		CY 1114019 T1	27 July 2016
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		EP 1707627 A4	27 August 2008
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		BR PI0417266 A	13 March 2007
		ES 2397631 T3	08 March 2013
		TW I343389 B	11 June 2011
		KR 101200188 B1	13 November 2012
		SI 1707627 T1	28 February 2013
		ZA 200605874 B	25 April 2008
		IL 176508 A	27 September 2011
		IN 200602744 P4	08 June 2007
		IN 201408287 P4	01 July 2016
		MX 289236 B	11 August 2011
		MX 2006006760 A1	01 October 2006

Form PCT/ISA/210 (patent family annex) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2018/124692

Patent document cited in search report				Publication date (day/month/year)		Patent family member(s)		Publication date (day/month/year)	
				SG	123452	A1	26 July 2006		
				SG	123452	B	31 July 2007		
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